

Technological Innovation and Industrial Relations

THE current bout of labour trouble in Britain, which among other things indirectly delayed the distribution of last week's *Nature* in North and South America and the Far East, is possibly the most serious since the Second World War. Most of the argument has come to centre on the question of whether the government has been wise in relying as much as it has done on the Industrial Relations Act as a means of persuading British workers and trade unions to follow explicit and novel rules in their dealings with employers. The act is by no means as horrendous as the unions say but there is an element of unreality in the government's belief that workers will instinctively comply with the requirements of an act they do not like and which is demonstrably unworkable in many of its parts—the recent strike which put the newspapers out of action, intended by the printing unions as a demonstration of support for the dockers and as a protest against the act itself, was frankly illegal under the new act, but it is exceedingly improbable that the unions concerned will be hauled before the Industrial Relations Court. In retrospect, no doubt even the government is wishing that it had sugared its pill by introducing the parts of the act which give workers a right to appeal against what they think are unfair dismissals, for example, before asking that trades unions should abandon their traditional if misguided ways. The row about the Industrial Relations Act, likely to continue for several weeks, may, however, obscure an even more interesting and important issue—the origin of the discontents of the past few weeks—the extent to which dock workers traditionally responsible for loading cargo into the holds of ships should acquiesce in the change in the pattern of dock labour brought about by the introduction of containers stuffed with cargo not at the docks but sometimes well inland.

In the past few years, British docks have been a recurring source of trouble. By the early 1960s, it had been recognized that handling equipment was out of date and that there were serious anomalies in the way in which a host of small companies made use of dock facilities at British ports for making profits from the loading and unloading of cargo vessels. Over the years, the fragmentation of the employers and the widespread practice of relying on casual labour had led to a needlessly meticulous and rigid definition of the kinds of jobs which dockers might perform and to a labour force which was at once too large and too insecure. In the past decade, successive British governments have fostered several schemes for the improvement of this unhealthy situation. The Dock Labour Scheme has the merit of giving most dock workers a measure of security—they are paid from a fund to which all port employers contribute even when there is no work for them, and there are also reasonably generous payments for redundancy. It would no doubt have been still better if the reorganization of the ports could have encouraged a still quicker amalgamation of the companies now operating at British ports, but what seemed until a few years to be a framework within which continuing

rationalization might be possible has been undermined by the widespread and rapid introduction of containers as a means of shipping cargo.

What is to be done? The first thing to be said is that it would be intolerable if anxiety among dockers about the spread of container transport should artificially restrict the uses which might be made of this invaluable and potentially economic means of transport. The truth is that the old-fashioned way of loading cargo into the holds of ships is entirely out of tune with modern thinking on production engineering. For is it not absurd that cargo intended for sea transport should be handled not merely at its origin and destination but twice at each of two intermediate ports as well? British dockers are at present asking that members of their union should have a right to employment at the centres at which containers are stuffed with goods but this is merely a side issue—and in the long run, a pointless issue. What everybody must recognize is that the decline of the dock labour force in the past five years should, on economic grounds, continue. Nobody will be surprised if there are merely 20,000 dockers working in Britain by the 1980s.

Situations like these are not unprecedented. A few years ago, in Britain, the coal industry was in a plight similar to that of the docks at present. In the end, successive governments decided to edge off the economic pressures on the coal miners and the communities of which they formed a predominant part by agreeing that the economic pressures on coal as a fuel should be artificially softened. The construction industry might have been in the same case if, by magic, factory-made buildings had not proved to be not merely feasible but economically attractive as well. What has happened to the British ports industry is that the pace of technological change has turned out to be faster than the speed with which the workers and the others who depend on the docks for a livelihood have been able to transform their way of life. It is unthinkable that nobody should accept responsibility for the consequences of such rapid change. The question, short of its trimmings, is who should pay. In countries such as Britain, there seems to be no doubt that ultimate responsibility must rest with the government, which is why it is sensible that the government should have agreed, last week, that it should pay for still more compelling inducements to persuade dockers to leave the industry. It remains an important question how best an attempt should be made to provide the workers for whom there will in future be no work with opportunities for changing their ways. Cash compensation is not enough. Should there not be a much more vigorous programme for the retraining of men like these in other crafts? The present government, like the past, for all its protestations about the value of and the need for retraining, has done less than it might to put flesh on the bones of an abstract concept. But the docks are the place to start, and urgently, for the need for imaginative retraining and redeployment will and should increase.



Counting the Chinese Population

To know how large the population of China is, and especially how quickly it is growing, is important because the population of China is so large. The Population Division of the United Nations estimated earlier this year that there are 760 million people in mainland China, just about 20 per cent of the estimated population of the world as a whole. But the estimates of the Chinese population are more seriously in error than those of any other major country. The United Nations modestly says that the true population in mid-1969 may have been anything between 732 million and 828 million.

The difficulty, of course, is the lack of accurate census data—the most recent census, itself an unconventional process by Western standards, was carried out as long ago as 1953. Given these uncertainties, it is no wonder that the Population Council of the United States has in the past few weeks excited great interest by its publication of a report on health and family planning services in mainland China by Dr Anibal Faundes and Dr Tapani Luukkainen of Santiago and Helsinki respectively (*Studies in Family Planning*, 3, 165; 1972). The two obstetricians spent two weeks in China in March this year and have now told a traveller's tale which has been widely interpreted as a sign that contraceptive devices of various kinds are now widely used in China and that, as a result, the immediate outlook for the growth of the Chinese population is more cheerful than has usually been supposed. Unfortunately, however, the necessarily anecdotal character of this report will prevent demographers from throwing their hats too high in the air.

Plainly, the government in Peking is as much at a loss as demographers elsewhere, for the decentralization of public administration means that only provincial and commune governments are in a position to monitor both the effectiveness of public health and the growth of the population within their parishes. There is apparently some hope that a more accurate picture of the demographic characteristics of the Chinese population will emerge from the registration of voters for the 1973 elections, but that is not a substitute for an accurate census. What Drs Faundes and Luukkainen have in the circumstances done is to provide estimates of birth and death rates in the cities of Peking and Shanghai which are themselves derived from information collected by the city governments. In Peking, for example, the annual birth rate is said to be 18.8 per thousand, and mortality 6.4 per thousand of total population, which corresponds to a rate of increase of 1.24 per cent per year. In Shanghai, a more western city, the birth rate is 12.08 per thousand and the mortality 5.2 per thousand, corresponding to a rate of increase of 0.69 per cent per year. These rates of increase are much lower than the estimate of between 1.9 and 2.0 per cent per year for mainland China as a whole. The difficulty is, of course, that the natural increase of an urban population in a developing country may frequently be much less than that of the surrounding rural population. Given the correlation between low infant mortality (easier to achieve in cities), the availability of education and personal prosperity and comparatively low birth rates, it is no surprise that city statistics are misleading.

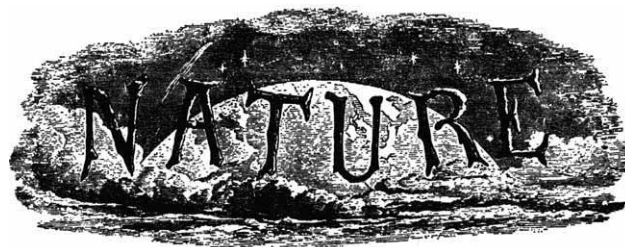
In the same spirit, what Drs Faundes and Luukkainen have to say about the practice of contraception in and

near Peking and Shanghai must also be taken with a grain of salt. The most striking part of their report is their declaration that 65 per cent of married couples in cities practise contraception and that in Shanghai no less than 89 per cent of married couples use some contraceptive method. It is extremely hard to believe this estimate—in the United States, for example, a survey completed in 1965 showed that 84 per cent of white couples had at some time practised contraception. In China, tubal ligations appear to be much more common than in the west. Abortions are easy to come by. Intrauterine devices are said to be more widely used in rural districts than in cities, where contraceptive pills appear to be more popular.

What does this imply? The only sure inference from the report by Drs Faundes and Luukkainen is that, in the cities at least, both the population and the public health authorities have heeded diligently the pleadings of the central government that the containment of population growth should be an important part of public policy. That is a hopeful sign, but much will depend on how quickly the rural areas will follow suit.

But nobody can be sure, which is why there is an urgent need that the government of China should organize a much better method of monitoring population growth than is available at present.

100 Years Ago



THE BRITISH ASSOCIATION

THE recurrence of our annual Congress of Science naturally leads us to reflect on the position which the British Association occupies in our social economy, and on the part it is qualified and, perhaps, destined to play. Whilst other scientific societies occupy themselves in giving publicity to results and speculations, and in rewarding with their medals successful labours, the British Association alone systematically undertakes to distribute the greater part of its income, about 2,000*l.* per annum, in grants to enable men of science to conduct scientific investigations, and to institute inquiries with a view to possible future action. Stated briefly, this constitutes the broad distinction between the British Association and other scientific societies. As a publishing society it cannot vie with some other bodies, as, for instance, with the Royal Society. The great bulk of the papers it receives are published in abstract and but few *in extenso*; and it allows a greater latitude than other societies with regard to the reception of subjects which have been elsewhere made public, thus constituting its proceedings, to a great extent, a *résumé* of the year's work—a characteristic quite in keeping with its practice of meeting but once a year.

From *Nature*, 6, 297, August 15, 1872.

OLD WORLD

Nuclear Power: Another Eighteen Months of Studies

AFTER twenty months of indecision while the government has reviewed its nuclear reactor policy, Mr John Davies announced this week that a further eighteen months of deliberation and study are to follow before firm decisions are taken on what type of reactor Britain is to build to follow the advanced gas cooled reactors (AGR).

Reaffirming the government's faith in both nuclear power and the Atomic Energy Authority, Mr Davies stated that while the government sees the fast breeder reactor as "the main element, in the long term, of our nuclear generating programme", it still intends to keep all its options open on which type of thermal reactor is to be built until the fast breeders become available in quantity, sometime in the 1980s. A complete and specific design and component development programme is to be commissioned at a cost of up to £6 million for the steam generating heavy water reactor, the five advanced gas cooled reactors under construction are to be completed "urgently", and possible design improvements are to be studied "with a view to maintaining the AGR as a possibility for future construction". Studies of the high temperature reactor are to continue, and the government is to seek assurance about the safety of the American light water reactors. "Within eighteen months" Mr Davies said, "all this work should have reached the stage where firm orders can be placed".

But if the choice of reactor type is to hang fire a major reorganization of the industry will take place soon. Mr Davies was guardedly unspecific as to what form the reorganization will take, but he did confirm that the industry will be consolidated into a single strong unit, closely involved with the AEA and British Nuclear Fuels Limited. The new group "should also have powerful technical and commercial backing", plus the ability to partake in European collaboration over the development and exploitation of nuclear reactors. Discussions with the interested parties will start immediately, Mr Davies said.

Further, a Nuclear Power Board is to be set up to advise Mr Davies on all aspects of nuclear power policy. "The board will have a major part to play in the decisions to be made in eighteen months' time about the ordering of generating plants." It is expected that industry, government, the AEA and the Central Electricity Generating Board will be represented on the board.

Although the further delay in deciding Britain's nuclear reactor policy in

detail will anger many people, Mr Davies's decision to launch a further eighteen months of studies is less surprising in the light of evidence Mr A. Hawkins, the recently appointed chairman of the CEGB, gave to the Select

Committee on Science and Technology last week. Mr Hawkins astounded the committee by informing it that the board only expects the demand for electricity to rise by between 3.5 and 5 per cent per annum over the next decade.

SELECT COMMITTEE

Much Ado About Everything

In a week of unprecedented activity, the Select Committee on Science and Technology tackled Lord Jellicoe about the government's White Paper on government research and development, discussed the future of the computer industry with Mr Christopher Chataway, visited the Atomic Energy Authority's steam generating heavy water reactor at Winfrith, took evidence from Mr A. Hawkins, chairman of the Central Electricity Generating Board (see above) and published its fifth report, this time on population policy.

Earlier in the session the select committee took evidence from Mr Robert Carr, Lord President of the Council and minister with responsibility for population, and from Sir Keith Joseph, Secretary of State for Social Services, to check on the progress being made towards the crystallization of a population policy—something that the select committee believes in passionately. Its report takes the government to task for "adopting a leisurely and disinterested attitude to the question of population growth", and reiterates the recommendations made in its first report on population in May 1971 that a special office be set up to advise the government on population policy, complete with a minister charged primarily with responsibility for such a policy. The committee also recommends in their latest report that comprehensive family planning services should be provided as a normal part of the National Health Service.

Lord Jellicoe had to face the select committee in one of its more belligerent moods. Aggrieved by the skimpy attention paid to its views in the White Paper, the select committee asked Lord Jellicoe why his reply to the committee's reports had been so cursory. Lord Jellicoe replied that the two paragraphs in the White Paper referring to the reports were not intended to be a full reply to the select committee. He said that detailed answers to the committee's arguments will be provided later, at the same time pledging the Government to a full debate on the White Paper in the House of Commons sometime next ses-

sion. With hindsight, Lord Jellicoe said, he felt that it would have been better if the White Paper had been introduced to the House with a statement; it might have prevented certain misunderstandings. The committee's views had been considered, however, and the White Paper had been held up at some inconvenience so that the committee's fourth report could be considered.

Questioned about the select committee's recommendation that government departments should produce an annual report on their research and development activities, Sir Alan Cottrell who accompanied Lord Jellicoe, said that he was looking into the ways in which this could be done and hoped to produce an answer by mid-August.

Lord Jellicoe informed the select committee that he hoped to receive an interim report on the interchange of scientists between government and academie from Professor Herman Bondi's working party by December.

The next day Mr Christopher Chataway, Minister for Industrial Development, disclosed to the committee that he is to have talks next month with the German and French governments about the future of the European computer industry. In the future, Mr Chataway said, there would probably only be one or two large computer companies in Europe, although at the moment he thinks that mergers between the larger companies would be very difficult. Nevertheless, he added, he hoped for closer cooperation in the short term and complete mergers in the long term.

Reaffirming the government's support for ICL, Mr Chataway explained that the £14.2 million of aid which the government had announced for the company was primarily to help it launch a new series of computers. The aid was by no means the whole cost of launching the range, and most of the money would come from ICL.

Mr Chataway went on to defend the single tender system for ICL. Single tendering is an important part of the government's aid for ICL, Mr Chataway said, even though in the long term the objective is to move away from it.

And the board considers the lower figure the more likely. If the growth rate does turn out to be 3.5 per cent a year, then the CEBG will need an extra 19,500 MW by the end of the decade. All but 4,000 MW of that have already been authorized, so that only three new stations will be required, only one of them nuclear. Even if demand does reach five per cent per annum Mr Hawkins said, the CEBG still only envisages four nuclear stations by 1982, and one of those will probably be a fast breeder. As a result Mr Hawkins informed the committee that the CEBG is hardly in a position to offer business to one nuclear consortium, let alone two as at present.

ENVIRONMENT

Lead Cut Lip Service

At long last Mr Peter Walker, Secretary of State for the Environment, has announced his proposals for reducing the lead content of petrol in Britain. The measures, first promised in December 1970, will lower the permitted levels from 0.84 g l.⁻¹ to 0.64 g l.⁻¹ by the end of this year, to 0.55 g l.⁻¹ by the end of 1973, and to 0.45 g l.⁻¹ by 1976.

In his statement in the House of Commons last week, Mr Walker said that "the Chief Medical Officer of Health has advised that the present levels of lead emission do not offer a danger to health, but that it is desirable that they should not be exceeded and they should if possible be reduced".

The reductions promised, however, will have little immediate effect (see *Nature*, 236, 104; 1972). Even though the permitted level of lead in petrol is 0.84 g l.⁻¹, the average level in 1971 was only 0.54 g l.⁻¹ and even five star petrol only had a lead level of 0.64 g l.⁻¹. Thus the new measures will have no effect until next year, and even then will scarcely make any difference to four star petrol (by far the best selling grade), which contained 0.55 g l.⁻¹ of lead in 1971.

The oil companies state that the reduction of lead levels will not affect the production of five star petrol even when the lead level is reduced to 0.45 g l.⁻¹ (the current lead content of three star petrol) as the octane rating can be raised by further refining; the full reduction proposed, however, should only raise prices by about 0.5p a gallon.

BRITISH ASSOCIATION

One Leap Ahead?

THE British Association for the Advancement of Science holds its 134th annual meeting in Leicester in September. Breaking with tradition, the meeting will last from Monday, September

4, to Saturday, September 9. There will be almost 250 lectures, forty films, five general symposia, and seventeen section meetings—not to mention the Science Fair, the section dinners and all the usual sideshows of this annual jamboree.

Among the more promising lectures on offer are Sir Vivian Fuch's presidential address on "Exploration—purpose and personality", Dr Kenneth Mellanby's "The biological effects of pollution—an ecological problem", and Dr M. J. Rees's Kelvin lecture on "Theoretical aspects of X-ray astronomy".

Other events that should prove informative and controversial are symposia on genetic engineering, life and leisure in the cities of today and the

year 2000, "Is science a menace?" and how science has been popularized. There is also an open forum on "Science Prospect '70s". Women's lib in science, diseases of the affluent society and the James report are all due for discussion, and if the words all prove too much, then participants can escape on one of the many excursions.

Behind the scenes, the association is planning yet another step into the twentieth century. The General Committee, the ultimate governing body, will be presented with a proposal that the council—the association's policy-making body—should be substantially reduced in size and that its members should be elected by the general membership, with the General Committee acting as a kind of electoral college.

CHESS

Spassky's Hopes Revive

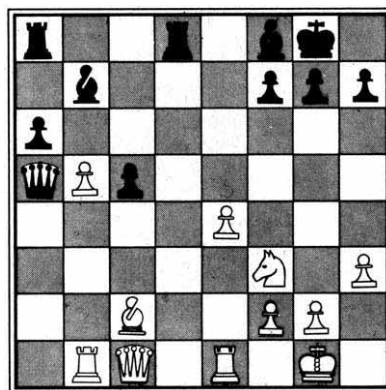
SPASSKY'S win in the eleventh game of the World Chess Championship is perhaps the break for which he has hoped. Fischer followed the same risky variation of the Sicilian Defence that he employed in the seventh game, but this time Spassky was much better prepared. The theme of white's game was the rather blatant attempt to trap Fischer's stray queen, and Spassky actually managed to achieve this by the twenty-fourth move. This win, although it still leaves Spassky trailing by 4½ to 6½ points, should at least encourage him to feel that the outcome of the match has not yet been settled.

The tenth game was strongly played by Fischer, particularly since Spassky was beginning to show greater truculence than in many previous games. Spassky answered Fischer's Ruy Lopez

with a system involving the subtle retreat of his queen's knight (9... N-N1) and a general regrouping of his queen's side formation. He boldly contested the centre by 17... P-B4 which allowed a series of near forced exchanges leading to the position in the diagram after 25... QxP.

Spassky probably considered that his position was quite satisfactory at this point and it must have been a shock to him when, after a series of systematic hammer blows by Fischer aimed at his king's bishop's pawn, he found himself obliged to surrender the "exchange" for a pawn. Note that 27... P-B5 would be answered by 28 BxP; and 30... K-R1 by 31... N-N6ch followed by mate. It is just conceivable that Fischer had even visualized the position arising after his thirty-third move (33 RxB) some time earlier, for instance when pondering over his seventeenth and eighteenth moves. The ensuing endgame proved to be without prospects for Spassky since he was unable to advance his queen's side pawns.—J.P.

SPASSKY BLACK



WHITE FISCHER

Position after Black's 25th move in the tenth match game

TENTH MATCH GAME

White: Fischer Black: Spassky

Ruy Lopez, Breyer Variation

White	Black	White	Black
1 P-K4	P-K4	29 QR-Q1	R-K2
2 N-KB3	N-QB3	30 BxP ch	RxB
3 B-N5	P-QR3	31 QxR ch	QxQ
4 B-R4	N-B3	32 NxQ	BxP
5 0-0	B-K2	33 RxB	KxN
6 R-K1	P-QN4	34 R-Q7 ch	K-B3
7 B-N3	P-Q3	35 R-N7	R-R8 ch
8 P-B3	0-0	36 K-R2	B-Q3 ch
9 P-KR3	N-N1	37 P-N3	P-N5
10 P-Q4	QN-Q2	38 K-N2	P-R4
11 QN-Q2	B-N2	39 R-N6	R-Q8
12 B-B2	R-K1	40 K-B3	K-B2
13 P-QN4	B-KB1	41 K-K2	R-Q4
14 P-QR4	N-N3	42 P-B4	P-N3
15 P-R5	N(N3)-Q2	43 P-N4	PxP
16 B-N2	Q-N1	44 PxP	P-N4
17 R-N1	P-B4	45 P-B5	B-K4
18 NPxP	PxBP	46 R-N5	K-B3
19 PxKP	N(Q2)xP	47 R(K4)xP	B-Q5
20 NxN	QxN	48 R-N6 ch	K-K4
21 P-QB4	Q-B5	49 K-B3	R-Q1
22 BxN	QxB	50 R-N8	R-Q2
23 PxP	KR-Q1	51 R(N4)-N7	R-Q3
24 Q-B1	Q-QB6	52 R-N6	R-Q2
25 N-B3	QxP	53 R-KN6	K-Q4
26 B-N3	PxP	54 RxB	B-K4
27 Q-KB4	R-Q2	55 P-B6	K-Q5
28 N-K5	Q-B2	56 R-N1	Resigns

NEW WORLD

Call for End to the Haldane Principle in Canada

by our Washington Correspondent

Ottawa, July

GOVERNMENT support for science and technology in Canada has, for most of this century, been dominated by the National Research Council of Canada, a unique institution concerned with the whole spectrum of scientific activities. Situated in several acres of land on the outskirts of Ottawa, the NRC disburses grants to universities, operates its own basic and industrial research laboratories, and until recently its executive committee had the chief advisory voice in the federal government on scientific affairs. In spite of a distinguished record, however, the NRC has come in for sharp criticism during the past few years, and it seems destined for some fundamental changes in its structure.

Critics of Canada's science policy have maintained that the country's industry lacks an adequate technological base, that too much of the national research effort is carried out in government laboratories and that there is a lack of synergism between the universities, industry and government (see *Nature*, 238, 242; 1972). Since the NRC has for so long been at the hub of the federal government's support for science and technology and since it accounts for nearly 20 per cent of the total government expenditure on scientific activities, it is only natural that the organization has been singled out for criticism from those who have suggested that all is not well with the management of Canadian science. But Dr William Schneider, President of the NRC, complained in a recent interview: "The NRC has been singled out for all of this, but this is grossly unfair".

Born during the First World War, the National Research Council has evolved into the embodiment of the so-called Haldane principle that government research and development should be the responsibility not of mission-oriented departments but of an independent agency. The NRC's executive committee reports, for example, to Mr C. M. Drury, President of the Treasury Board, a Cabinet Minister who has no other direct responsibility for science and technology, and its activities fall within the province of most departments of the federal government. The council now spends about \$140 million a year, about a third of which finds its way into the universities for research support,

SCIENCE IN CANADA

This is the second report from our Washington correspondent.

another third is spent on running the NRC's own laboratories, and the rest is divided between postgraduate student support, grants to industry and administration. Virtually all the expenditure is derived from parliamentary appropriations.

It is the council's independence from mission-oriented departments that has drawn the sharpest fire from its critics and, once again, the chief catalyst for the public debate over the council's role has been the report of the special Senate Committee on Science Policy, under the chairmanship of Senator Maurice Lamontagne. The Science Council of Canada, which took over the NRC's role as chief adviser to the government on science policy, also believes that changes in the NRC's structure are required, and Dr Omond Solandt, who retired as director of the Science Council in June, fired a strong parting shot across the bows of the NRC in his last annual report.

The most likely change in the council's structure would be to split the university granting functions away from the NRC laboratory operations. The Lamontagne Committee has recommended such a move and the Science Council has suggested that "most members of the Science Council would generally agree with the Senate Committee's recommendations that the NRC's university research granting function be separated from the laboratory function". Dr Solandt also argues for the establishment of a single granting body to take over the university support functions of the NRC, those relating to basic research from the Medical Research Council (the second largest single source of university research funds from the federal government) and the support of social sciences and humanities from the Canada Council.

The chief argument for integrating the present councils is to provide more effectively for multidisciplinary research, and to circumvent the possibility that worthwhile grants may not be given under the present arrangement because they do not fall into the pro-

vince of any of the existing granting bodies. Dr Solandt also argues that splitting off the NRC's granting functions may be beneficial for the council's industrial research laboratories. He suggests that although the laboratories were established to provide support to industry, their close association with the universities through the granting mechanisms has given them "traditionally a more academic than industrial orientation".

What difference would it make to integrate the existing councils and separate off the NRC's granting functions? Not much, according to some observers. For one thing, there is already *de facto* separation of the NRC's functions since, as Senator Lamontagne points out, the NRC's board devotes 90 per cent of its time and attention to the distribution of awards and grants. But the Senate committee believes that closer integration of the three chief grant-awarding bodies may facilitate giving priorities to specific areas of research—it suggests that in the 1970s, the social sciences should be given the biggest push, followed by the life sciences. The physical sciences, on the other hand, which have traditionally dominated science spending in Canada, would go into a relative decline if the senators had their way.

The chief source of opposition to all this is, of course, the National Research Council itself, which stands in danger of being dismantled. Dr Schneider, for example, suggests that the Senate committee's proposals would simply increase the gap between the universities, industry and the government. He pointed out that "the NRC is an agency that is now straddling the three sectors and it is an agency that can be used for building the bridge". Similarly, Dr C. C. Butler, Director of the NRC Division of Biological Sciences, said that although from his point of view as a laboratory director it would be convenient to get the granting committees out of the way, the everyday interaction between the members of granting committees, basic and industrial scientists is beneficial for all concerned.

If the granting functions are taken away from the National Research Council, what would happen to its laboratories? The Senate committee and Dr Solandt agree on one thing—that the curiosity-oriented research groups in the council should be considered

separately from the mission-oriented research groups. Dr Solandt suggests that the pure research groups should be organized into an institute of pure research, reporting to the NRC council, and that there should be a regular interchange of staff between the institute and the universities. And the Senate committee recommends much the same thing.

As for the applied research laboratories, the chief criticism directed against them is that they have become divorced from their original mission of helping industry. According to Dr Solandt, "one could argue endlessly about the pros and cons of their very existence. To be pragmatic, I take the view that they do exist, that they do represent an investment and a resource and that they can be so organized as to make an effective contribution to our national development". His recipe is chiefly to have the National Research Council report to the Department of Industry, Trade and Commerce, a move which should ensure that there is closer relationship between the council's objectives and those of the department.

For long the National Research Council has been widely praised, especially outside Canada, and last year it achieved its crowning glory when Dr Gerhard Herzberg, who works at the Council's Ottawa laboratories, was awarded the Nobel Prize for Chemistry. But it seems that the very features for which it has often been praised—its integration of basic and applied research and of university granting functions—have, according to its critics, caused it to become too much of an ivory tower.

Mr Alastair Gillespie, Minister of State for Science and Technology, in whose hands the fate of the National Research Council probably rests, is keeping silent for now. "I have not encouraged speculation about the NRC—this gets people upset for longer than is necessary", he said recently. Nevertheless, Gillespie is believed to be considering changes in the council, and it is likely that if the Liberal Party survives the forthcoming elections (which will probably be held in the autumn), the NRC may figure in the new government's plans for next session.

MEDICAL ETHICS

The Rights of the Subject

by our Washington Correspondent

DURING the past two weeks, details have been revealed of a 40-year long study, started in the early 1930s, of the effects of latent syphilis on some 400 blacks in Alabama. It has been alleged that the subjects of the study were never informed that they had syphilis, and that although penicillin has been available for treatment of the disease since the early 1950s, medical treatment has been

deliberately withheld. The story, first published by the Associated Press, has raised a public outcry, initiated a formal inquiry by the Department of Health, Education and Welfare, and sparked off renewed interest in protecting the rights of human subjects in biomedical research.

Less than a week after the story broke, the Senate passed a measure designed to prevent such a study ever taking place again. The unlikely vehicle for passage of the measure was the Military Procurement Authorizations bill, to which Senator Edward M. Kennedy successfully attached an amendment whose applications extend far beyond the confines of defence research. The salient part of the amendment reads:

It is hereby declared to be the policy of the United States that Federal funds may be used to conduct research involving human beings as experimental subjects only when each participant has freely volunteered to participate after having been fully informed of any physical or mental health risks which may be incurred as a result of participating in such research.

APPOINTMENTS

Plugging the Gaps

by our Washington Correspondent

THERE has recently been a flurry of appointments announced by the White House to fill long-standing vacancies on top level science advisory committees. During the past two weeks President Nixon has announced the appointment of four new members of the President's Science Advisory Committee (there have been three vacancies since January 1 this year), the nomination of eight people to be members of the National Science Board (to fill positions vacant since May) and the appointment of nine members of the President's Committee on the National Medal of Science. Described in a White House press release as "the highest recognition offered by the Federal Government for distinguished achievement" in the physical, biological, mathematical, or engineering sciences, the National Medal of Science has not been awarded since May last year, and that was for 1970.

New members of PSAC are Luis W. Alvarez (University of California, Berkeley), Gerald F. Tape (President, Associated Universities, Inc.), Howard S. Turner (Turner Construction Co.), James B. Wyngaarden (Duke University). Nominees to the National Science Board are Wesley G. Campbell (Hoover Institute on War, Revolution and Peace), T. Marshall Hahn, jun. (Virginia Polytechnic Institute and State University), Anna Jane Harrison (Mount Holyoke College, Mass.), Hubert Heffner (Stan-

ford University), William H. Meckling (University of Rochester, NY), William A. Nirenberg (Scripps Institute of Oceanography), Russell D. O'Neal (Bendix Corporation) and Joseph M. Reynolds (Louisiana State University).

DES

Banned at Last

by our Washington Correspondent

THE Food and Drug Administration has at last decided to ban use of the synthetic hormone diethylstilboestrol (DES) for fattening sheep and cattle in the United States. The FDA announced last week that production of DES for animal feeds must be halted immediately, although existing stocks of the hormone can be used until January 1 next year. The ban, which is a reversal of the agency's past policies (see *Nature*, 238, 67; 1972), has been instituted because fresh evidence has come to light which suggests that residues of DES cannot be prevented from turning up in meat.

The evidence which precipitated the ban has come from a radioactive tracer study which shows that residues of DES remain in tissues of cattle for at least seven days after the animals have been taken off treated feedlots. Because the hormone is a very powerful carcinogen, US food and drug laws prescribe that it must not enter the food supply. The FDA had previously allowed it to be used in animal feeds on the assumption that none will remain in the tissues of animals which have been taken off treated feeds for a suitable length of time before slaughter.

The study which shot holes in the FDA's policy was carried out by the Agricultural Research Service, an agency of the Department of Agriculture. Six steers were fed 10 mg doses of DES twice a day—the usual doses used by cattle farmers—to establish a feeding pattern, and they were then fed a single dose of ¹⁴C labelled hormone. DES residues were found in their livers at levels of up to 0.5 parts per thousand million seven days later.

Why have previous tests failed to uncover the flaw in the FDA's regulatory policies? According to an official of the Agricultural Research Service, the recent tests, which used ¹⁴C labelled DES, are much more sensitive than previous ones which used deuterium labelled hormone. The carbon labelled compound has only recently become available. Moreover, the bioassay which was used until recently in the Department of Agriculture's testing programme was not sensitive enough to pick up residues in animal livers below about two parts per thousand million, a level that allowed livers contaminated with small amounts of DES to slip through. That essentially is what the FDA's critics have argued all along.

NEWS AND VIEWS

Death and Dominance of an Antibody

WHEN a scientific method is first described its potential is not always realized; some technical advance which seems to have only intrinsic value is often the progenitor of a battery of articles. One such step, which may be just starting to be exploited in this way, is that which was first described in 1970 by B. A. Askonas and her colleagues at the National Institute for Medical Research, Mill Hill (*Proc. US Nat. Acad. Sci.*, **67**, 1398; 1970). In this report Askonas *et al.* described a method for the cloning *in vivo* of cells which form a single species of antibody.

This method involved transferring only a very few cells from the spleens of mice immunized with dinitrophenylated bovine gamma globulin (DNP-BGG) into irradiated syngeneic mice and then assessing the degree of antibody heterogeneity by isoelectric focusing. Thin layers of polyacrylamide gels in which the various species of antibody had been separated were coated with ¹³¹I-labelled antigen so that the individual antibody bands could be visualized with autoradiography. Antibody in the serum from one of the recipient mice did, in fact, produce the sort of isoelectric pattern which might be expected if only a single type of cell was producing antibody and it proved possible to pass the spleen cells of this mouse, E9, to other mice, whereupon the cells continued to produce this same antibody. Although the production of E9 was entirely dependent on the administration of antigen it did not seem to matter whether this was given *in vivo* or whether the cells were treated during their passage *in vitro*. In the absence of antigen the cells remained viable *in vivo* for at least four weeks and the antigen presumably triggered the cells into division and therefore increased the numbers of E9-antibody forming cells.

Cells could be passaged in this manner for a period of about six months but, as with all good things, E9 antibody production ultimately came to an end and (apart from those in cold storage) the cells were finally lost. Antibody forming cells therefore seemed to have a finite life span. Although the nicety of this technique is in itself sufficient justification for the work, a couple of questions were raised which must have plagued the authors, for they are now discussed in more detail by Askonas and her colleague A. R. Williamson on pages 337 and 339 of this issue of *Nature*.

Williamson and Askonas think that the inability to transfer and prime antibody forming cells continuously may be a case of "senescence *in vivo*", and although it is pointed out that the cell clone might be eradicated if there were insufficient T cells to act cooperatively during antibody production there is one experiment which makes this unlikely. Injection of additional primed spleen cells into those animals in which E9 cells were being passaged did not increase the maximum concentration of antibody, although this would have provided numbers of T cells far in excess of those required. The decline in production can therefore hardly be attributed

to limitation by inadequate numbers of helper cells.

The effect which the physiology of irradiated mice might have on transplanted cells is not considered and it is to be admitted that ageing and death is a much more attractive concept. Indeed, if the system should really prove to be an example of senescence *in vivo* then a means is provided for measuring both the division potential and the functional capacity of a single clone of cells, and the system might well prove to be of use in evaluating some of the various immunological theories of ageing. In fact, although the evidence is as yet only circumstantial it must gladden the hearts of those gerontologists who belong to the "differentiate and die" school, for Williamson and Askonas believe that the senescence of these cells is the direct result of their differentiated nature. Whether they are right or not remains to be seen, but the system can be expected to become an attractive alternative to that of diploid fibroblast culture *in vitro* as a tool for the study of ageing.

An even more intriguing, although unrelated, question which the system poses is discussed by Askonas and Williamson on page 339. Why is only a single species of antibody produced throughout the life span of these cells? The lymphoid tissues of the recipient mice will gradually recover from the effects of irradiation and antigen reactive cells other than those of E9 might be expected to accumulate. Again, when normal or primed spleen cells were inoculated to replace a possible deficiency of T-derived helper cells it is certain that antibody precursor cells must have been present, but only the E9 antibody continued to be produced. Good evidence for the presence of other antibody precursor cells is in fact provided, for the dominant and inhibitory effect of E9 was only evident when the concentration of circulating antibody was high; once it fell below a certain value heterogeneous antibody was produced.

It is possible to explain this phenomenon by suggesting that there is competition for antigen between the circulating antibody and receptors on the memory cells. E9 antibody production would not be turned off because it would be only those cells with a low affinity for antigen, lower than that of the circulating antibody, which would avoid being stimulated. But if this system were in operation there would be continuous selection for antibody with the highest affinity and, because it is only when the levels of E9 fall that antibody with a higher affinity is detected, this suggestion is not valid.

The alternatives revolve around ideas which suppose that the immature antigen reactive cells are more sensitive to switching off, or that they have fewer receptors, or that the presence of circulating antibody may affect the handling and presentation of antigen to the immature cells, but they are suggestions which need more definitive evidence to support them.

This observation and the ideas concerning its origin would seem to be very relevant to other immunological problems, particularly those of tolerance and "blocking"

and it seems fairly safe to predict that it will be in these fields that the cloning technique will prove most useful.

Although it may be some time before either of these lines of re-

search is fully developed, it is clear that the technique for cloning and propagating single antibody forming cells is not one which should be readily forgotten.—From our Cell Physiology Correspondent.

Late Pleistocene Man at Kow Swamp

Thorne and Macumber's interim report on the late Pleistocene population from Kow Swamp in Australia (see page 316 of this issue of *Nature*) raises various questions, some new and some hardy perennials. First of all, anthropologists should consider whether the material—which is fragmentary—is representative of a general stock as opposed to perhaps a small local, and not generally typical, inbred community. Second, it must be asked whether the robustness of the remains is to some extent an artefact of the differential survival of the bones after burial—stronger and thicker bones being more likely to survive intact. Another problem is whether it is reasonable and meaningful to suggest direct-line evolutionary affinities between *Homo erectus* and a late Pleistocene group—separated perhaps by half a million years. Furthermore, is it possible to make tentative evolutionary sequences between a modern and late Pleistocene population, keeping in mind that the Pleistocene precursors of any modern population could have been markedly different?

At all times during hominid evolution there has been an ever changing mosaic pattern and it is difficult to know how to assess the "total morphological pattern" of a fossil in comparison with others. Clearly not all morphological features have the same taxonomic value. Yet how can weighting be introduced without the possibility of bias in interpretation? In the case of the Kow Swamp material, for instance, the frontal and facial regions in particular recall the morphology of certain Middle Pleistocene hominid fossils of half a million years ago, but it is another matter whether these features deserve special emphasis in any attempt to assess the evolutionary affinities of the group.

One could extend these questions to other more specific matters. What, for instance, is the significance of cranial thickness? Has it any

taxonomic value or is it purely indicative of a nutritional status or a low grade anaemia? Because the Kow Swamp skulls were not grotesquely thickened, the significance of the vault thickness really depends on precise studies on the whole question of the reasons for varying cranial thickness in any population (? genetic + endocrine + nutritional + pathology).

A constantly occurring problem, which again is raised by the Kow Swamp article, is the nature of the measurements used. Although there has been a move in recent decades to tailor the measurement to fit the skeletal investigation, there is still the same need as ever for internationally agreed and standardized measurement. This is particularly the case with fossil hominid material, where there are plenty of published measurements but quite a few are poorly defined or not strictly comparable. In the Kow Swamp material, some biometricians may question exactly how the supraorbital breadth, postorbital constriction or ramus height were taken. In the case of cranial thickness, although Thorne and Macumber have every right to give measurements near sutural margins, it seems likely that these are not

the best areas to use in assessing thickness, and some methodological revision is needed here. Because human palaeontology is resorting more and more to multivariate statistics, clear definitions of basic measurements seem even more essential.

Whatever the eventual accepted interpretation of the Kow Swamp people, the evolution and interrelationships of Upper Pleistocene populations in South-east Asia and Oceania remain a more complex problem. If the 40,000-year-old Niah skull from Borneo can be relied upon, there is evidence of an advanced and apparently modern *Homo sapiens* form probably contemporary with a robust, large browed group as seen in the Javanese Solo people—who may well be an advanced *Homo erectus* "remnant" population. The circumstances which gave rise to such a marked divergence of forms in Asia are not known, nor is there much fossil evidence to indicate the degree of intermixing which may have occurred between such varieties once evolved. It is not even known to what extent the advanced forms gave rise to the mongoloids or were the precursors of such apparently non-mongoloid groups as the Ainu of Japan and the Australian aborigines.

It is certain, however, that during late Pleistocene times in the eastern Asiatic area there must have been the emergence of various small communities who were mixed to some degree (as in the great variety of European/Negro groups in the world today). The earliest Australians

DNA Transcription and Replication

FURTHER evidence that the replication of DNA in *Escherichia coli* depends directly on the transcription of some part of the DNA that is to be replicated is reported next Wednesday (August 16) in *Nature New Biology* by Messing, Staudenbauer and Hofschneider. Several groups have shown that the replication of the *E. coli* chromosome is blocked by antibiotics which specifically inhibit transcription, but such data do not prove that transcription *per se* rather than protein synthesis is essential for DNA replication.

To prove that it is transcription, rather than a requirement for a messenger RNA that can be translated, which is essential for DNA replication, Messing *et al.* studied the effect of rif-

ampicin and of two inhibitors of translation, chloramphenicol and puromycin, on the replication of minicircular plasmid DNA in *E. coli* 15 THU.

They found that this covalently circular, double stranded and supercoiled DNA is replicated in the presence of the two inhibitors of translation, whereas these drugs inhibit replication of the *E. coli* chromosome. Rifampicin, however, blocks the replication of the *E. coli* chromosome and also the replication of the minicircular plasmid DNA. The conclusion must be, therefore, that replication of this plasmid DNA depends directly on transcription and presumably that is also true of the replication of the *E. coli* chromosome.

therefore may not have been a homogeneous group, and indeed the variation which was clearly evident in the Australians and Tasmanians during the nineteenth century, before decimation by European disease and "administration", gives support to the idea of a heterogeneous mosaic in these populations.

As far as Kow Swamp is concerned, anthropologists will look forward to publication of a detailed report and to the availability of casts for comparative study. For the present the biological status of this prehistoric Australian group will remain somewhat controversial.—From a Correspondent.

POLYSACCHARIDES

The Forgotten Molecules

from our Molecular Biology Correspondent

In an era in which biochemistry, for better or worse, is dominated by macromolecules, the polysaccharides have been more or less lost to view. Most biochemists would probably admit if pressed that they regard their colleagues in the carbohydrate field as a race apart, plying their lugubrious trade in dignified isolation, far from the bustle of molecular biology. Of late Rees and his associates have done much to narrow the gulf by showing that some at least of the polysaccharides possess an unsuspected conformational versatility in solution, and are capable of interactions and transitions that can be described in language comprehensible to those who have grown up with nucleic acids and proteins.

One knows, of course, from X-ray studies that ordered structures exist in the solid state, but in solution the evidence is circumstantial. In polymers like the carrageenans and agarose, gelation is accompanied by optical changes. In the case of κ -carrageenan, in particular, these have been interpreted in terms of the conversion of randomly coiled chains into interrupted double helices. From a number of physical and chemical lines of evidence, Rees and his colleagues inferred that the non-covalent cross-links, which are required for gel formation, depend for their existence on the presence of helix-interrupting kinks in the chain. These kinks have been chemically characterized, and are subject to specific hydrolytic scission. The resulting fragments undergo the physical changes normally associated with gelation, without, however, generating any gel. In particular the order-disorder transition is accompanied by the two-fold change in molecular weight, which is expected to result from strand separation, when the double

helices melt. The double helix, it may be noted, is also the structure deduced from X-ray analysis of fibrous κ -carrageenan.

Now Dea, McKinnon and Rees (*J. Mol. Biol.*, **68**, 153; 1972) have shown that specific conformation-linked interactions can occur in solution between polysaccharides of different kinds. Agarose, like the carrageenans, displays a change in optical rotation, betokening the breakdown of an ordered structure, when gel melts, and this effect is fully reversible, though with a huge hysteretic lag, amounting to some 50°. The galactomannans, on the other hand, do not gel, and show no optical transitions. These molecules consist of a mannan backbone, with single galactose units grafted on at intervals, the tendency being for the galactose-substituted residues to occur in blocks. In the solid state these polymers are extended chains, and so, Dea *et al.* suggest, are they in solution. When mixed with the non-gelling fragmented κ -carrageenan the gelling capacity reappears.

The same phenomenon is observed in mixtures of the galactomannan with fragmented agarose, and the galactomannan will also provoke gelation in solutions containing intact agarose at concentrations below that at which it will gel by itself. The greater the density of galactose substitution in the galactomannan the lower is its ability to form such mixed gels. The formation of helices in the mixtures is inferred both from the changes in optical rotation and the behaviour of the dye, methylene blue, which acts as an empirical indicator of conformation: it evidently interacts with the ordered, presumed helical, state of the polysaccharide, and this inter-

action elicits large changes, characteristic of the formation of dye stacks, in the visible absorption spectrum, as well as the onset of induced Cotton effects in the intrinsically optically inactive chromophore.

Dea *et al.* have a model for the interaction of the polymers. On the grounds that galactose substitution inhibits association with the helices of carrageenan or agarose, it is postulated that it is the blocks of unsubstituted residues that combine in extended form with the helices, the uncombined galactose-rich segments then functioning as cross-links. In the agarose complex the optical rotation undergoes a change in the course of gelation, which is in the opposite sense to that associated with the coil→helix transition of agarose alone. This suggests that there is an overriding contribution of the opposite sign from a conformational change in the galactomannan. This interpretation is borne out by re-heating experiments, in which the very large hysteresis that characterizes the agarose transition allows a partial separation of the contributions of the complex and of the uncomplexed agarose. Rees has formulated empirical rules which relate optical rotation with backbone torsional angles, taking into account restrictions imposed by model building. Application of these rules to the contribution of the galactomannan in the complex structure permits an enlightened guess at its possible conformation in this environment.

Dea *et al.* offer finally some cogitations on what they conceive to be the biological relevance of these interactions. The unsubstituted parts of the galactomannan they regard as a model for the rather similar structural poly-

Stratospheric Warmings in the Southern Hemisphere

STUDIES of the southern hemisphere stratosphere may be aided as a result of an investigation carried out by Eric L. Unthank of the University of Melbourne and reported in next Monday's *Nature Physical Science* (August 14). Unthank has found that measurements taken at an observing station near Melbourne can give an indication of conditions in the stratosphere between Australia and Antarctica. This discovery is helpful, as one of the obstacles to a better understanding of the southern stratosphere is the sparsity of observing stations.

In particular Unthank is interested in those occasions known as stratospheric warmings, which occur when the circulation of the winter stratosphere is disrupted and the stratospheric temperature rises for several weeks by as much as several tens of degrees. He has compared observations of the stratosphere made from a

station near Melbourne with observations taken 1,300 miles to the southeast at Macquarie Island.

The measurements show that the temperature of the stratosphere about 30 km above Macquarie Island correlates with the stratospheric winds at about 30 km above the observing station near Melbourne. When temperatures are low above Macquarie Island there are westerly winds above the mainland station, and high temperatures above Macquarie Island occur with easterly winds above the mainland station.

This result is a step towards the reduction of the disparity between what is known of the northern and southern stratospheres. The extent of the correlation between the two widely separated stations should throw further light on the extent of stratospheric warmings and associated effects in the southern hemisphere.

mers, cellulose, hemicelluloses and the peptidoglycan of the bacterial cell wall. They refer, moreover, to observations of an interaction between galactomannan and a bacterial polysaccharide. These relationships, they suggest, must play a part in determining the organization and the mechanical properties of cell walls and other structures.

PROTEIN SYNTHESIS

Interchangeable Factors

from our Molecular Genetics Correspondent

THE universal nature of the genetic code and the ability of the protein synthetic apparatus of one type of organism to translate the messenger RNAs of another species *in vitro* point to the development of the code as a very early stage in evolution. The sole differences between the dictionaries of code words for amino-acids in different organisms seem to be confined to the punctuation signals; the AUG codon which initiates protein synthesis responds to a formylated methionyl-tRNA in bacteria but to a methionyl-tRNA with a free amino-group in the cells of higher organisms. The termination signals are probably the same in all organisms, although there is some evidence which suggests that the UGA codon may be used for the amino-acid cysteine in eukaryotes.

This common evolution is further emphasized in an article from Zasloff and Ochoa (Proc. US Nat. Acad. Sci., **69**, 1796; 1972) which shows that the protein factors which initiate protein synthesis are interchangeable between a variety of organisms. In *Escherichia coli* cells, there are three such factors; f3 which seems to direct 30S subunits of the ribosome to bind to initiation sites on messenger RNA, f2 which assists the binding of fmet-tRNA_i to this complex, and f1 which seems to play some part in both processes. These factors can be washed off 30S subunits with ammonium chloride and counterparts have been found in some eukaryotic cells; in other higher organisms, the proteins needed for initiation are found in the supernatant and do not seem to be associated with the ribosome subunits.

Zasloff and Ochoa have previously isolated a protein factor from the brine shrimp, *Artemia salina*, which seems to correspond to factor f2 of *E. coli*, the only obvious difference in its action being that it does not require GTP for its activity. The factor has a molecular weight of about 100,000—which is much larger than its bacterial analogue—and shares with f2 a sensitivity to the reagent N-ethyl-maleimide which reacts with free sulphhydryl groups. Zasloff and Ochoa have now prepared a mixed system which contains the ribosomes

of the shrimp, but in which they substitute the initiation factors of mouse L cells, rat liver, or rabbit reticulocytes in place of the *Artemia* factor. They find that these heterologous systems work perfectly well; and so does the reverse exchange system in which the *Artemia* factor promotes binding of the initiator tRNA to the ribosomes of rat liver. The eukaryotic factors cannot be replaced by bacterial factor f2, nor can they replace f2 with bacterial ribosomes. But the interchangeability of the factors from these different eukaryotes indicates that not only have the signals which start proteins been conserved in evolution, but also the mechanisms which respond to them.

Another heterologous initiation complex has been formed by Noll, Noll and Lingrel (*ibid.*, 1843); they have assayed the ability of the ribosomes, initiator tRNA, and initiation factors of *E. coli* to form complexes with mammalian globin messenger RNA. These complexes are readily formed, and the bound formyl-methionine can be released by puromycin, which indicates that it enters its usual binding site on the ribosome. The first amino-acid—after the initiator methionine which is later removed—in both globin chains is valine; if the ribosomes are binding to the correct initiation sites, therefore, valyl-tRNA should be bound to the complex. But although binding takes place with a mixture of aminoacyl-tRNAs and amino-acids can be incorporated into protein, valyl-tRNA does not bind specifically.

This implies that the ribosomes have formed an initiation complex at the wrong site on the messenger. But binding seems to be specific and has the same characteristics as binding to the

correct initiation sites on messengers used in *E. coli*. This suggests that the sequence on mRNA to which ribosomes bind may be longer than the AUG codon itself and is different in *E. coli* and mammals; the sequence to which the *E. coli* ribosomes bind on globin mRNA must fortuitously resemble the natural initiation sites in *E. coli* messengers.

This is important for two reasons. First, it confirms the idea that AUG codons within a message may be distinguished from those which act as initiators by their context; the nature of this context may be different in prokaryotes and eukaryotes, perhaps because in the first mRNA is translated even as it transcribed from DNA, whereas in the cells of higher organisms the completed messenger must be transported from nucleus to cytoplasm.

Even more interesting is the possibility that the binding site on the globin mRNA can be sequenced by the now tried technique of degrading the mRNA in the complex with ribonuclease; the sequence bound to the messenger is protected and can be recovered and sequenced. The chief problem in using this technique is simply lack of sufficient messenger; so far, only the binding sites of RNA phages have been determined and these have not thrown much light on the initiation process, perhaps because these molecules are not true cellular RNAs and represent a special case. Globin mRNA can be prepared in large amounts and it will be intriguing to see whether there really is only one sequence on mammalian mRNA to which ribosomes bind or whether there is less specificity. And one important caution which these results point to is that specific binding in

Neuraminidase Stimulates Cell Division

A VARIETY of factors—including serum, proteases, insulin and, of course, tumour viruses—can induce confluent monolayers of cultivated cells, which have ceased to divide, to begin further rounds of cell division. And because proteases immobilized on beads are as effective as enzyme in solution it seems likely that at least some of these stimulatory factors exert their effects by causing changes in the chemistry and/or architecture of the cell surface. In next Wednesday's *Nature New Biology* (August 16), Vaheri, Ruoslahti and Nordling add another enzyme, neuraminidase, to the list of molecules capable of triggering multiplication in confluent cultures of cells. That neuraminidase has this effect is not surprising because sialic acid is a component of cell surface glycolipids and glycoproteins which are attacked by this enzyme.

Vaheri and his colleagues found that neuraminidase from either *V. cholerae* or *Cl. perfringens* induces DNA synthesis and mitosis in confluent cultures of chick fibroblasts to about 30 per cent at most of the extent obtained with optimal amounts of insulin. Stimulation of cell division by neuraminidase was proportional to the concentration of the enzyme over the range 0.02 µg/ml. to about 0.5 µg/ml., and at these concentrations no protease activity could be detected contaminating the neuraminidase. Vaheri *et al.* also measured the uptake of 2-deoxy-D-³H-glucose and report that after exposure to neuraminidase it is enhanced by some two to three-fold. They suggest that all these data indicate "that sialic acid containing membrane molecules are critical for growth control and for the transmission of the stimulatory effect of neuraminidase, insulin and trypsin".

heterologous systems is apparently not equivalent to proper initiation; they imply that binding assays are no substitute for characterizing the protein product and showing that the protein product is that coded by the gene.

COMMUNITY ECOLOGY

Patterns in Rain Forest

from a Correspondent

Two articles by Janzen (*Ecology*, **53**, 258 and 350; 1972) assist in evaluating the role of animals in determining the spatial patterns and numbers of species (diversity) of trees in lowland rain forest in tropical America.

Where insects have evolved the habit of feeding on only one or a few species of plant, different insects tend to select different plants. It has long been recognized that the number of such insect species which can coexist in a community must in part depend on the number of plant species present. The converse, that plant diversity may be increased by the activities of insects, is a more recent idea.

Janzen proposes a model in which seeds and seedlings of a rain-forest tree are eaten by species-specific predators which, because of their specificity, occur in populations centred on each seed-producing tree. The density of seeds declines with distance from the parent tree in a manner dependent on the method of seed dispersal, and the probability of survival of individual seeds and seedlings increases with distance from the parent and the predators associated with it. The probability of maturation of a new tree is thus close to zero both near the parent and far from it, with a peak at some intermediate distance. If this distance is great and the probability of maturation low, then the equilibrium density of mature trees is low. Very many tree species with this type of population structure can be accommodated in a community, and Janzen argues that this model, with modifications, may account for the regular (non-clumped) distributions of lowland neotropical trees and for the high tree diversities recorded from rain forests.

This hypothesis can be tested and refined by measuring seed distributions and spatial patterns of germination, maturation and predation for individual populations of several tree species. Two such studies broadly corroborate the model.

In a Costa Rican deciduous forest, Janzen found that seeds of the tree *Sterculia apetala* are eaten by the bug *Dysdercus fasciatus*. Immature seed pods are cut open by parrots and squirrels and the seeds are eaten. Later, as the pods begin to split, smaller birds and *Dysdercus* can gain access. Janzen

noticed that populations of *Dysdercus*, perhaps founded by individuals which had survived on trees fruiting out of synchrony with the majority, built up so rapidly that seeds falling under the parent tree were attacked within a few minutes, whereas those placed (experimentally) at distances of 60 m or more were very unlikely to be discovered. The seed pods contain stiff hairs which adhere to the bodies of arboreal mammals, and reproduction of the tree apparently depends on the dispersion of mature pods by these mammals, who open them and drop some of the seeds.

In a second study on the island of Puerto Rico where seed-eating vertebrates are scarce, palm trees (*Euterpe globosa*) were found to have a fruiting season which is strictly synchronized between individual trees. This enables early seeds to escape predation while the density of seed-eating scolytid beetles builds up. Later, most of the seeds were eaten but not before many

seedlings had become established around the parent trees. This palm thus occurs at a much higher density than that typical of rain-forest trees. Where this type of situation is widespread, one would expect the tree species composition of the forest to be dependent more on competitive relationships between plants and less on the nature of specific plant-insect interactions.

TOXICOLOGY

Mercury Intoxication

from a Correspondent

AGAINST the background of anxiety about hazards from hard metals, several features of mercury intoxication were described at a colloquium organized by the Biochemical Society at the University of Surrey, Guildford, on July 20.

The first part of the programme dealt with the effects of mercury in the

New Data on Interstellar Gas

THERE has recently been much interest in the properties of interstellar gas, one of the reasons for this research activity being that knowledge about interstellar gas is a necessary precursor to reliable theories about star formation.

Many types of observation can be used to study interstellar gas; two of the relevant parameters are the average electron density and the electron temperature. For the immediate neighbourhood of the Sun it is possible to use a combination of observations such as the difference in arrival times of pulsar signals at different frequencies or the relative intensities of the spectral lines of neutral and ionized calcium, combined with data on free-free absorption and emission. From these data Pottasch has concluded that near the Sun there are electrons in both low temperature ($T \sim 100$ K) low ionization clouds as well as in high temperature ($T \sim 12,000$ K) high ionization clouds; he arrives at an average electron density of between 0.04 and 0.1 cm^{-3} .

There are, however, several indications that conditions near the centre of our Galaxy are different; these cannot be studied by pulsar measurements, for instance, because pulsars can only be observed if they lie within a few kpc of the Sun. But for a study of conditions within, say, 7 kpc from the centre of the Galaxy there is the powerful method of observing various hydrogenic recombination lines at radiofrequencies. Provided the assumption can be made that the interstellar gas is in local thermal equilibrium, one can derive from the observed intensities both the electron density and the electron temperature. Earlier

measurements of this kind arrived at an electron temperature of the order of 1,000 K. Next week in *Nature Physical Science* (August 14), R. D. Davies, H. E. Matthews and A. Pedlar report their observations of the 166α line in several directions in the plane of the Galaxy.

The measurements give the intensity of the line in a given direction as well as the position of the line; from the Doppler shift of the line, as compared with its position in the laboratory, it is possible to find the region in space where the line originates, for 21 cm line data provide a distribution of gas velocities as a function of the distance from the Sun in a given direction.

Davies *et al.* observed that the recombination line emission is strongest in the central region of the Galaxy, that is, at distances less than about 7 kpc from the centre, whereas the emission from the neighbourhood of the Sun or, in general, from other regions more than 8 kpc from the centre of the Galaxy, is at least five times weaker. They also find that the electron distribution is patchy with typical sizes of the emission regions of the order of 1 kpc; the same patchiness had earlier been observed in free-free emission studies.

From the observed intensities, Davies *et al.* conclude that the electron density is about 10 cm^{-3} in the emitting regions and that the electron temperature is about $6,000 \pm 2,000$ K, appreciably higher than earlier derived values. It is interesting to note that the extensive H II regions found by the Jodrell Bank observers in the Galaxy are very similar in size and properties to the extended H α regions observed in the inner parts of many other spiral galaxies.

whole animal and factors affecting the distribution of mercury in its different forms because such knowledge is essential for a proper understanding of the basic hazards presented by mercury in the environment. Dr P. Lesley Bidstrup (London) emphasized that poisoning caused by inorganic and aryl mercury compounds (for example, phenylmercury acetate) is quite dissimilar to that caused by short chain alkylmercury compounds (for example, methylmercury derivatives). Chronic poisoning with inorganic mercury compounds reveals well-defined neurological symptoms such as erethism and tremor from which recovery is complete when exposure ceases; however, poisoning with short chain alkylmercury compounds presents a bizarre neurological picture, as described by Dr D. Hunter, after industrial exposure and observed in large scale outbreaks of poisoning in Iraq and Japan during the past twenty years. Dr W. H. Butler (MRC Toxicology Unit, Carshalton) described the neurological lesions involved in poisoning by methylmercury compounds where damage is chiefly in the cerebellum and sensory pathways with lesions in the cerebral cortex in man. This damage involves the entire sensory pathway and is distinct from that caused by organophosphorus compounds in which sensory nerves die back gradually from the periphery. Dr Butler also emphasized that both organo and inorganic mercurials produce renal tubular damage.

Dr T. W. Clarkson (University of Rochester) suggested that because many proteins have thiol groups which are potential acceptors for mercury compounds, only studies of the uptake, distribution and excretion of the various forms of mercury would eventually explain the different effects of mercurials. Methyl- and phenylmercury compounds are efficiently absorbed from the gastrointestinal tract whereas inorganic mercury is not. Moreover, the biological half-life of methylmercury is considerably longer than that of inorganic and arylmercury compounds. This is associated with a persistent entero-hepatic circulation of methylmercury. Studies of this nature depend, of course, on methods for discriminating between forms of mercury, and Dr L. Magos (MRC Toxicology Unit, Carshalton) reviewed the methods which he has developed.

Dr J. W. Daniel (ICI, Alderley Park) then spoke of the cleavage of organomercury compounds to inorganic mercury in mammalian systems. The widely used fungicide, phenylmercury acetate, can be degraded to inorganic mercury and phenol (by way of benzene) in the cytosolic fraction of rat liver by an NAD(P)H independent

mechanism. By contrast, methylmercury compounds are only slowly cleaved to inorganic mercury in rats. A mercury-resistant pseudomonad can also convert phenyl-, ethyl- and methylmercury to metallic mercury and benzene, ethane and methane respectively in the presence of NAD(P)H and thioglycollate.

The widespread distribution of methylmercury in lakes and oceans results from methylation of inorganic mercury. Dr L. Landner (Swedish Water and Air Pollution Research Laboratory, Stockholm) spoke in detail of the methylation of inorganic mercury by *Neurospora crassa* by way of a vitamin B₁₂ independent pathway which does not involve transmethylation with S-adenosyl methionine as methyl donor. Furthermore, this alkylation of mercury carried out by resistant microorganisms must be considered a detoxification process be-

cause newly formed methylmercury is rendered harmless to the organisms by its binding to homocysteine.

Turning to the action on isolated systems, Dr M. J. Selwyn (University of East Anglia) described the catalysis by organomercurials of anion transporting systems across biological and artificial membranes. Such a phenomenon is in contrast to the actions of organomercurials as non-specific inhibitors of thiol-dependent enzymes and transport processes. Furthermore, this catalytic process is not confined to organomercurials but is also exhibited by organo-tin and organo-lead compounds which are also of toxicological interest. These compounds are therefore potentially useful not only in studying exchange processes but also in appreciating the underlying mechanisms by which such toxic molecules are taken up by certain tissues.

Myelin and Conduction Velocity

ACCORDING to the ionic theory of nervous conduction, impulses in nerve and muscle fibres are initiated by a local depolarization of the cell membrane which causes a regenerative entry of sodium ions leading to a full-sized action potential. Propagation depends on the passive spread of current ahead of the active region of the membrane, which in turn triggers a full-sized response, such that impulses are transmitted in an all-or-none fashion, without decrement.

The speed of impulse conduction depends therefore on the rate at which the membrane ahead of the impulse is depolarized beyond the threshold level. This in turn is determined by the electrical properties of the membrane and the longitudinal resistances of the media inside and outside the axon. If all other factors are assumed to be equal, the conduction velocity would be expected to increase with the square root of the fibre diameter. This relationship seems to be followed in invertebrates, the giant axons of the squid being a clear example of the achievement of high speed through increased fibre size. In vertebrates, however, where economy of space occupied becomes important, a compromise has been reached through the development of the medullated axon, in which the myelin sheath acts as an insulator which restricts the active generation of current to the sites at which the sheath is interrupted at the nodes of Ranvier, thereby increasing conduction velocity by forcing the local currents to act at a considerable distance ahead of the active region.

The factors which influence conduction velocity become more complex when myelinated fibres are considered; but Rushton, in a classical paper (*J. Physiol.*, **115**, 101; 1951), derived certain

interesting relationships using a combination of mathematical theory with data quoted from experimental observation of other workers. Theory alone predicts that, for fibres with similar specific membrane properties, an optimum thickness of myelin exists such that the ratio of the axon diameter to the overall fibre diameter is about 0.6; too thin a myelin sheath would not provide adequate insulation, and too narrow an axon core would result in a high resistance to current flow. This seems to hold true for a range of fibres from the central and peripheral nervous systems, but in a report in next Wednesday's *Nature New Biology* (August 16) S. G. Waxman and M. V. L. Bennett dispute Rushton's second principal conclusion.

Rushton claims that conduction velocity in myelinated fibres can be shown to be proportional to the diameter D , rather than $\sqrt{D}$ as in non-myelinated fibres, and that it follows therefore that the smallest nerves will conduct faster if non-myelinated, the largest if myelinated. On this argument, the transition size where myelination first begins to pay off is around 1μ . This is in fact the size where fibres of the peripheral nervous system are first found to acquire their sheath, but, as Waxman and Bennett point out, in the central nervous system, axons as small as 0.2μ may be myelinated. They claim that Rushton's argument is erroneous chiefly on the grounds that he derives his relationship between conduction velocity and diameter by putting into a theoretical equation values for g , the ratio of axonal to total fibre diameter, derived from morphological data which do not apply either to central or peripheral fibres.

A Curriculum Development Project at University Level

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This article deals with curriculum development at university level, and is based largely on Mr Dowdeswell's experience as coordinator of the Inter-University Biology Teaching Project (IUBTP), which has involved cooperation between five universities.

To justify curriculum development its necessity must be demonstrated, either from pressure by students or by teaching staff, or from a change of climate within a particular area of learning—frequently a combination of all three. Time spent at this stage is valuable, for preliminary negotiations may result not only in a clearer statement of aims, but also in a more effective strategy for achieving them. Thus an initial request from the Zoology Department of Queen Elizabeth College for help in reviewing curricula and teaching methods, and instituting changes where necessary, evolved, under the sponsorship of the Nuffield Foundation, into a project with broader aims involving five universities.

This article is chiefly based on my experience, over the past two and a half years as coordinator of the Inter-University Biology Teaching Project, involving cooperation between Bath, Birmingham, Glasgow, London (Queen Elizabeth and Chelsea Colleges) and Sussex. The organization and activities of the project have been described elsewhere^{1,2}; basically, its overall aims have been to study new methods of biology education at university level, with particular reference to the part that independent learning can play, and to develop materials appropriate for this purpose. Because of the limited time and resources available, the new materials are restricted in their scope, and should be regarded as a set of educational models, their average duration being about 15 h. Exceptions are those produced by Sussex which are about double this length. Each module has been closely linked with appropriate laboratory and tutorial work.

At the outset we attempted to identify those areas of learning where such restricted contributions might be most useful and our enquiries suggested that three different kinds of materials were likely to be needed. Thus Bridge courses are intended to span the gap between knowledge acquired at school and that needed for first year university work; Technique courses deal with the acquisition of essential skills which tends to demand repeated demonstration by a lecturer, wasteful both in time and manpower; Main courses are modules covering "key" topics, and can be used at any stage in a university course where they may be needed. The range of products of the Inter-University Biology Teaching Project summarized by categories is shown in Table 1.

Team, Timing and Finance

Although the number of individuals and range of interests represented in the preliminary stages of justification should

be as large as possible, the size of the group responsible for developing the materials should be quite small—between two and five. Specialized knowledge or expertise not available within the group can then be called in as required and arrangements made for appropriate remuneration. Varied outlooks and attitudes are certainly desirable within a team, but they must not be large enough to retard creative thought and activity by increasing the possibility of friction or stalemate. Above all, if the project and its products are to be taken seriously, it is essential that the team should be headed by a senior member of the department concerned, preferably seconded full time. Not only will he be needed to establish good public relations both within the department and with the outside world, but he will also have to assemble and edit the materials, a process requiring an experienced hand unhampered by lack of time.

In spite of the considerable fund of experience now available from the various Nuffield projects and elsewhere, there is still a tendency to underestimate the time needed for the completion of a piece of curriculum development. This is possibly due to the mistaken impression that the appearance of the new materials in trial form marks the end of the project. In fact, it indicates only that the half-way stage has been reached—if that. Validation and rewriting in the light of the feedback from trials can be a lengthy process, particularly at university level. We have found that the completion time for such projects is between 3 and 4 years. In the IUBTP the limited time has meant that we have had to confine ourselves chiefly to the production of a limited range of teaching materials, to the exclusion of some of the larger issues that have emerged in the process. These include the relevance and organization of laboratory work, overlapping between subjects, and the justification for including particular topics in the curriculum, to mention only a few. In such critical areas of teaching the educational technologist could have a valuable part to play in the future, helping to isolate problems and to evolve new methods for their solution.

An axiom of good curriculum development is that it cannot be done cheaply. The funds made available to the IUBTP by the Nuffield Foundation amounted to £80,000 over two years; it has in fact proved possible to support the project in three universities for a third year. The project

Table 1 Products of the Inter-University Biology Teaching Project

University	Title of module	Type of module
Bath	Aseptic technique in microbiology Growth of microbes	Technique course Bridge course
Birmingham	Growth and structure of flowering plants Electricity for biologists	Bridge course Bridge course
Glasgow	Developmental biology Animal behaviour	Bridge course Bridge course
London	Enzymes Genetics	Main course Bridge course
Sussex	Introductory biology for physical scientists	Bridge course

was concerned principally with the development of self-instructional materials; for some universities, this involved the production of varying amounts of films and slides, and hence the setting up of the necessary technical facilities for producing them. This aspect may well have inflated expenditure in a few instances somewhat beyond what might be regarded as the average. As with all projects, however, by far the most expensive item was salaries, which amounted in different universities to between 60 and 80% of the total spent. As indicated earlier, when budgeting for a project it is important to bear in mind the likelihood of incidental expenses; specialists' salaries, organization of meetings and conferences, unforeseen demands for sets of trial materials and so forth. In our case, a sum of £15,000 was set aside as a reserve fund, of which about £8,000 was spent in the first two years—more than half of this being on the salaries of a part-time secretary and a staff replacement for the co-ordinator who was seconded to the project on a half-time basis.

Development of Materials

The sequence of events in developing new curricula has been well documented³⁻⁵, but I shall, however, describe briefly the different phases of the operation, chiefly from an organizational standpoint.

Although the broad aims of a project will no doubt have been determined at the outset, the translation of these into more objective terms within an overall framework can be difficult and protracted. In drawing up learning objectives—indicating changes in knowledge and attitudes expected of students as a result of experiencing each section of the course—there is bound to be an interaction between the initial aims and the teaching materials eventually chosen to achieve them. This means that the final statement of objectives in operational terms is usually not possible until the development of the materials themselves is partly complete.

In the design of a new curriculum it is obviously important to have as clear a picture as possible—background knowledge, attitudes, interests and so forth—of the students for whom it is intended. Much of the material produced by the IUBTP was intended to bridge the gap between secondary school and university, so it might have been supposed that the possession of the appropriate A-level passes would have provided sufficient evidence of intellectual background. But this was not so, and additional pre-testing was found to be necessary in order to avoid making erroneous assumptions.

Some existing university syllabuses in biology showed that the grounds for including particular contents were often far from rational. The notion that in devising a curriculum it is desirable at the outset to establish aims before deciding on content is evidently quite new to many university departments. As explained already, the project in fact comprised five separate university sub-projects mostly further subdivided, and each with somewhat differing aims and objectives. Broadly speaking, our aims were, first, to provide new teaching materials in biology at university level, particularly in relation to the first undergraduate year, and, second, to investigate ways in which the teaching and learning of biology could benefit from the contributions of modern educational technology and methods of curriculum development.

One of the principal justifications for our decision to concentrate on a series of modules based largely on independent learning was that, although there was some evidence from the United States^{6,7} and elsewhere that such materials are appropriate at university level, virtually no attempt had so far been made to try them out in Britain. It was also clear from available evidence that certain kinds of biological materials would probably lend themselves well to treatment in this way. For instance, in the study of genetics and development there appeared to be a need for short

“bridging” courses to help span the gap between knowledge acquired at school and that needed for the first year university work.

We also felt that topics chosen carefully and presented in modular form should have the advantage of flexibility and relevance to a wide variety of circumstances. A typical example is a module on enzymes which could be used at several levels and in the different contexts of botany, zoology, biochemistry and microbial genetics.

Independent learning modules have the obvious advantage that relatively little briefing of university tutors on teaching methods is required⁶⁻⁸. Some of the most complex problems may eventually prove to be organizational; for instance when a restricted range of materials has to be shared between many students. We hoped that if good use were made throughout the new materials of modern methods of presentation and learning, some of these methods would eventually also find their way into more traditional university teaching⁹.

Presentation and Production

In a department which is well endowed with a particular educational facility, for instance for the making of 8 mm film loops or 35 mm slides, there is inevitably a temptation to use it exclusively in curriculum development and this may lead to a stereotyped product. In the development of a new course, the broad aims and objectives leading to the selection of appropriate materials must be established before decisions are made on how they should be presented. But the two stages must not be regarded as separate entities for, as thinking proceeds, there is inevitably some degree of interaction; thus the mode of presentation may well have an appreciable effect on the shaping of objectives in their final form.

Projects like IUBTP can provide unique opportunities for learning more about suitable methods of presentation through trying and comparing several different methods simultaneously. Lack of time, however, militates against this by stepping up pressures on production, and requiring a final decision on media to be made before the necessary research can be completed.

The production of new materials making use of the wide range of learning media now available demands, among other things, a full range of photographic apparatus with technicians to operate it. Such facilities are expensive and take time to establish. In the IUBTP, each university made its own local arrangements and, in retrospect, a good deal of time and money might have been saved had we pooled resources and set up, say, two conveniently sited and properly equipped centres. But more crucial still was the acute shortage of personnel with a knowledge of educational technology and its application in curriculum development. At present there appears to be a national deficiency in this field and, if it persists, it could well hamper the development of curriculum studies. The terms of our agreement with the Nuffield Foundation committed us to the eventual publication of our products, and this only served to exacerbate the problems outlined above, for the standard of audio-visual materials acceptable for publication is high.

Post-testing, validation and rewriting are, in a sense, the most crucial phases in a curriculum development programme, for if they are carried out inadequately there is no other way of gauging the success of the project. Proper validation can be a lengthy process, and, under university conditions, where the availability of groups of students for use in trials is apt to fluctuate with changing departmental timetables, at least one year needs to be set aside for the purpose. Where feedback indicates that extensive re-writing is needed, it may be necessary to institute further trials of the materials in modified form. Planners are sometimes curiously reluctant to accord sufficient priority to validation,

perhaps because of a feeling that the process is somehow "unproductive". But in adopting such an attitude they overlook an important secondary role of trials—that of dissemination. The wider the range of testing the greater will be the body of informed opinion on which the adoption of new materials and methods will ultimately depend.

Ideally, we would have liked to test the validity of our modes of presentation against similar material presented in a more traditional manner. Time has, however, precluded any such research, and validation has had to be restricted largely to comparisons of student performance by means of pre- and post-tests.

A curriculum project stands or falls by its public relations both with the immediate circle of those involved and with the outside world. In this respect, one of the problems facing the IUBTP was that in spite of a certain amount of advance publicity, few members of university departments had much idea of the purpose of the undertaking. As a result, demands by teams for such facilities as office space, time for discussions and seminars with students, and groups of undergraduates for trials, tended at first to be met with suspicion if not outright hostility. The speed and degree of success with which these problems were overcome varied in different universities. The problem was essentially one of achieving an identity as quickly as possible, and some of the chief factors contributing towards success were the establishment of a headquarters office (with an appropriate sign outside!), the development of good personal relations with other members of the department and the laboratory staff, and the provision of information on the progress and purpose of the project in a readily assimilable form, for instance by the issue of brief news sheets. It has already been stressed that a senior member of the department should be involved in the project, for he can influence greatly the attitude of the rest of the staff. He should have sufficient conventional status (in terms of research publications and administrative experience, as well as teaching ability, for example) to exert some authority, and although involved he should retain his administrative responsibilities so as to remain well informed on wider university matters.

Communication within the higher education sector generally has also presented considerable problems, and although each university has forged links of its own through trials, personal contacts and limited publicity, we have yet to find a satisfactory means of disseminating widely information relating to the project as a whole. About half-way through the project each university held a number of "open days", and a one-day symposium was arranged in the rooms of the Royal Society to which representatives of biology departments in all universities and polytechnics were invited. The occasion provided an opportunity for a preliminary discussion and a display of all materials, but the impact on the biology teaching community as a whole was inevitably rather small. Judging by the reported commercial success in the United States of a programme somewhat similar to one of ours, it may well be that in the end, the most effective publicity will be that organized by the publishers on our behalf or in collaboration with us.

All curriculum development is bound to be expensive because, apart from consumables, it requires the employment of highly skilled people. Such investment therefore needs to be justified by results in terms of extensive consideration and use. To achieve this outcome in the future, some measure of inter-institutional collaboration would seem to be essential. Since the first products of the IUBTP have yet to be published, it is too early to make predictions of the extent to which they may be adopted.

The success of a curriculum development project must be judged primarily by its tangible products. The IUBTP has also achieved significant intangible successes, however, which, in the long term, may well prove to be some of its most important contributions to biology teaching. In parti-

cular, it has undoubtedly stimulated in a number of British universities an increased awareness of the importance of teaching methods and a realization that new techniques of learning such as self-instruction have much to offer, both when used alone and also in combination with more traditional procedures. In some universities, new thinking has proceeded a stage further and has led to a more critical appraisal of existing teaching programmes. Another important byproduct of the project has been to alert universities to the need for greater cooperation, and to the advantages that can derive not only from the pooling of technical resources, referred to earlier, but also from the broader range of outlooks that can be brought to bear on common problems of curriculum development.

Mention has been made before of the extensive use of independent learning methods in the United States, both at university and school levels. Indeed, a widespread ferment of activity is now resulting in the production of great quantities of modular, self-instructional materials many of which will no doubt appear on the British market before long. This prompts the question whether some sort of international cooperation would not be advantageous so as to avoid an excessive duplication of effort, such as is already occurring in the United States. On the face of it, the idea certainly seems worth pursuing, possibly along the lines of setting up an international clearing house or information centre. To be effective, such an organization would require considerable funds for the purchase of sets of materials and to provide the necessary facilities for inspecting them. Previous experience has shown that any such establishment which relies for its contents solely on the charity of publishers is likely to be inadequately stocked and hence to purvey misleading information. Above all, a comprehensive system of cataloguing would be needed so that the materials could be accurately classified in terms of quality, intellectual level, and the degree of validation to which they had been subjected. While it is misleading to attempt to draw too close a comparison between the educational systems of different countries, it is roughly true to say that the first year of American College corresponds to our secondary school Sixth Form. Thus, modules produced for first year undergraduates would be unlikely to suit our universities except, perhaps, as Bridge courses. As yet, hardly any of the mass of materials developed by American universities and schools have been validated outside the institutions for which they were designed. In this respect, the procedure adopted by the IUBTP of extending validation as widely as possible, may well have established a valuable pattern for the future.

I thank Mr R. A. Becher of the Nuffield Foundation and members of the Executive and General Purposes Committee of the Inter-University Biology Teaching Project for many helpful comments and suggestions in the writing of this account.

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Discoveries of Late Pleistocene Man at Kow Swamp, Australia

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The late Pleistocene human remains from Kow Swamp display archaic cranial features which suggest the survival of *Homo erectus* in Australia until as recently as 10,000 years ago.

THIS article describes the first extensive collection of late Pleistocene human remains from Australia. Analysis of the cranial morphology of more than thirty individuals reveals the survival of *Homo erectus* features in Australia until as recently as 10,000 years ago.

In August 1967, a highly mineralized and carbonate encrusted partial skeleton with archaic cranial features was examined during a survey of the skeletal collections held by the National Museum of Victoria. The rest of this skeleton (KS1) was excavated during 1968¹ from silts bordering Kow Swamp in northern Victoria (Fig. 1). Subsequent excavations in this area have produced the remains of at least forty individuals. Burial areas at two nearby sites, Gunbower and Bourkes Bridge, contain material of similar morphology but as yet have not been explored in detail.

At Kow Swamp there is a distinct concentration of burials near Taylors Creek. Thirteen graves have been excavated and many burials remain to be investigated. The construction of an irrigation contour channel through part of the site disturbed at least twenty burials. Reconstruction of these disturbed skeletons is simplified because differential mineralization has rendered each individual a different colour. The population includes infants and juveniles. At present fifteen adult individuals are complete enough for detailed description.

Most of the skeletal material is the result of shallow, primary burials into relatively soft lacustrine and aeolian sediments. Preservation has been enhanced by carbonate mineralization, generally with encrustation up to 10 mm thick. Orientation of cadavers included full extension, crouching and tight flexion. Stone artefacts, ochre, shells and marsupial teeth were placed in some graves. At least one individual had been cremated. (Evidence of cremation is not surprising, in view of its presence in Australia at 25–32,000 years BP².) A gritty shoreline deposit adjacent to the KS2-17 site yielded more than fifty quartz artefacts, many of them carbonate encrusted.

Geology

All the sites lie on the Riverine Plain in Victoria, astride a major flow path of the ancestral Murray River system and its

tributaries—a path that has been in existence since late Miocene times. The association of burials with ancestral rivers indicates general occupation of this area in the late glacial period when the streams had much greater discharges than at present^{3,4}.

Physiographically, the sites fall into two groups—those associated with an ancient Kow Swamp shore line (KS1-17), and burials in the levees of a former stream system, a distributary of which (Mead Stream) once flowed past the northern edge of the swamp. The Cohuna Cranium⁵, discovered in 1925, stems from the second group, as do the Gunbower and Bourkes Bridge burials (Fig. 1).

Kow Swamp Group

Kow Swamp is at present a largely artificial reservoir which occupies the depression of an earlier late Quaternary lake formed in a back levee position to the ancient Mead Stream⁶. Around the eastern edge of the swamp is a narrow belt of lacustrine silts (Cohuna Silt), about 0.25 km wide and only 1 m thick. The Cohuna Silt is in part overlain by the Kow Sand which forms a low crescentic dune (lunette), rising to a height of about 4 m above the plain. Skeletal material occurs in both the Cohuna Silt and Kow Sand.

(a) Cohuna Silt sites. In this area, two distinct sites have yielded portions of about thirty-five individuals, of which twelve were recovered from undisturbed graves. These graves had been dug from the present (albeit fossil) surface. The silts are a near-shore lacustrine deposit which marks the eastern limits of the former Lake Kow. They wedge out to the east against the rising underlying clay plain but lakewards are underlain by lacustrine fine sands. The presence of unionid shells throughout the entire sequence indicates freshwater conditions during deposition. The silts were later infused by carbonates of groundwater origin. Subsequent remobilization of the carbonate has led to a dynamic, highly calcareous environment which provides optimum conditions for bone preservation. In some instances grave margins have been preserved by a continuous layer of carbonate, precipitated along the junction of the grave fill and the undisturbed silts.

(b) Kow Sand sites. The lunette commences south of Taylors Creek and rises abruptly to 4 m. In this region skeletal material was obtained from two out of three pits dug at random—one contained the undisturbed KS9 skeleton at a depth of 1.4 m. The grave of this individual is the only one dug from an old surface, which was subsequently covered by continuing dune accretion. The lunette partially overlies the Cohuna Silt, with no major intervening palaeosol to indicate a significant

hiatus between the two units. Elluviated and illuviated zones subsequently developed in the dune, the event coinciding with a phase of carbonate elluviation, pan formation and bone encrustation in the exposed silts. Bone preservation in the non-calcareous Kow Sand is poor, as lithological and pedological factors favour its erosion. Near the KS9 site, for instance, all that remained of a shallow burial were two small pieces of femur.

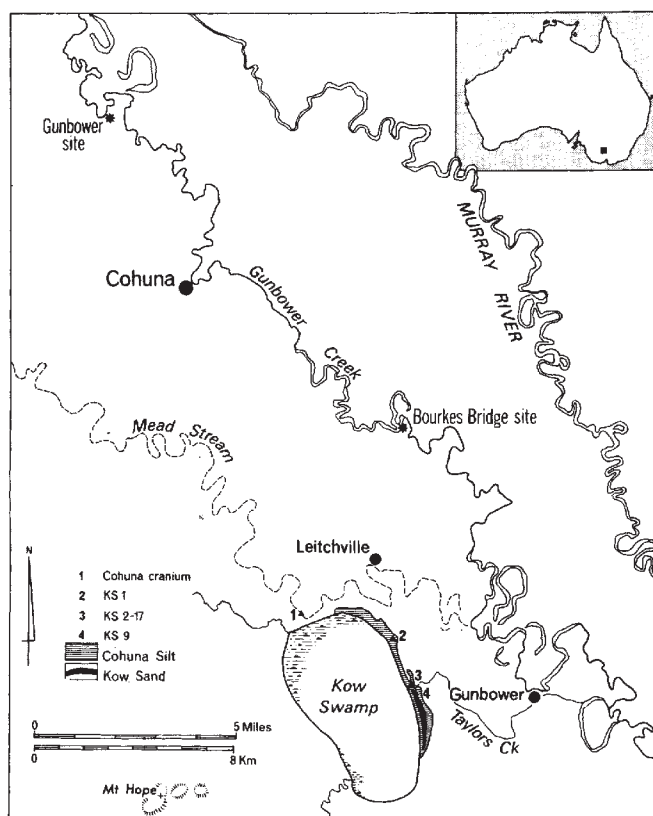


Fig. 1 Distribution of burial sites in the Kow Swamp area.

Levee Group

The Cohuna Cranium site is situated near Kow Swamp on levees of the late Pleistocene Mead Stream. The Gunbower and Bourkes Bridge sites are in similar sediments adjacent to Gunbower Creek, although their positions suggest occupational affinities with the ancestral Gunbower Creek rather than the older Mead sediments in which they are buried. The Gunbower and Bourkes Bridge skeletons are the result of shallow burials into sandy silts—at Bourkes Bridge a section shows 1.3 m of carbonate impregnated silts above a clay base. As at Kow Swamp, calcareous zones have developed within the profiles, and the skeletons have a protective crust of precipitated soil carbonate (at Bourkes Bridge a skeleton was embedded in a

carbonate pan 25 cm thick and had to be removed in blocks). The positions and pedological similarities of all these sites suggest a relatively limited time range for the burials.

Chronology

Radiocarbon dating was carried out on charcoal and bone collected from lacustrine and aeolian environments, along a distance of 2 km (Table 1). The dating is being undertaken as a cooperative project with H. A. Polach (Radiocarbon Laboratory, Australian National University).

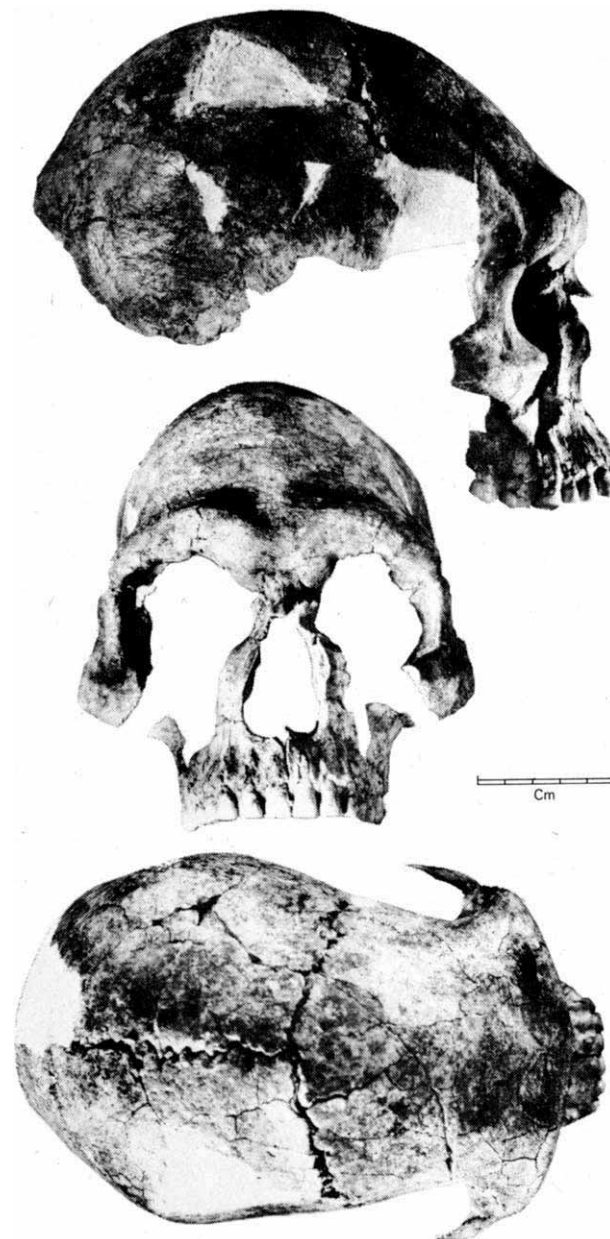


Fig. 2 Three views of the KS1 cranium.

Table 1 Kow Swamp Radiocarbon Dates

Laboratory sample No.	Location and material	Age (years BP)
ANU-403b	KS1, bone apatite	10,070 ± 250
ANU-533	Shoreline (?) grits adjacent to KS1-17 site, charcoal	9,260 ± 270
ANU-532	KS9, fragmented charcoal	9,590 ± 130
ANU-531	KS9, fragmented charcoal	8,080 ± 420
ANU-619b	KS9, bone apatite	9,300 ± 220

Only one charcoal sample (ANU-533) has been dated because their shallowness from the silts poses sampling problems arising from possible contamination by bush fires. The two lunette samples were collected beside the KS9 skeleton and overlapped stratigraphically. The lower of the two samples gave a slightly younger date of 8,080 BP (ANU-531), which may reflect rootlet contamination. The upper sample (ANU-532) and the bone apatite date (ANU-619b) are virtually

identical. The KS1 apatite date is not yet confirmed by charcoal samples. In spite of these variations the dates are consistent; they indicate that the Kow Swamp population occupied the ancient Lake Kow shoreline from at least 10,000 BP until after 9,000 BP.

Cranial Morphology

The Kow Swamp crania are large and dolicocephalic, with thick vault bone and well developed muscle insertions. They display a complex of archaic characteristics, particularly in the frontal and facial regions, not seen in recent Aboriginal crania. Major cranial measurements are shown in Table 2.

Table 2 Measurements of Kow Swamp Crania (in mm)

Measurement	Range	Number
Glabella-opisthocranium length	193-216	8
Maximum breadth (biparietal)	126-140	8
Supraorbital breadth	110->138	9
Postorbital constriction	22-35	9
Bizygomatic breadth	130->155	6
Frontal curvature index *	12.5-19	10
Nasion-prosthion	72-80	4
Nasion-bregma	118-126	6

* Based on nasion-bregma chord.

The major vault bones are uniformly thick, the diploic and tabular proportions being normal. Thicknesses are: at bregma (9-11.5 mm), midfrontal squama (8.5-13.5 mm), lambda (8-11.5 mm), mid-parietal (8-10 mm), parietal at asterion (8.5-11.5 mm).



Fig. 3 The cranium of KS5.

The frontal bones exhibit marked recession, one of the most striking features of the series. It was suspected that the low frontal curvature index values might represent a sex difference, but KS3, a female, has a value of 14.6. The range (12.5-18) overlaps the ranges for near contemporary Aboriginal crania (17.3-31.7) and *Homo erectus* (12-18.6). The males have massive supraorbital regions, with a thick and prominent glabella leading laterally to well developed superciliary ridges and thickened zygomatic trigones. A true supraorbital torus is

present in five specimens. All crania possess supraglabella fossae, expanded laterally into distinct ophrionic grooves. In KS8 and KS9 the groove is equivalent to that in the La Chapelle cranium. The frontal squama is either flat, passing into a low prebregmatic eminence, or runs in a low smooth curve to bregma and beyond. The poorly filled temporal regions produce a high degree of postorbital constriction.



Fig. 4 Mandibles of KS5 (top) and KS1.

The dorsal outline shows slight to moderate gabbling of the vault. There is no evidence of a depressed sagittal suture, nor of parasagittal depression. The degree of prominence of the parietal eminences is slight to moderate. Viewed laterally the outline of the parietals is a smooth curve passing into a mild but distinct obelion depression.

Table 3 Measurements of Kow Swamp Mandibles (in mm)

Measurement	Range	Number
Total length	111-118	3
Bicondylar breadth	138-145	2
Bigonial breadth	116-125	3
Height at symphysis	36-39	4
Height at M1-M2	33-37	3
Ramus height	72-77	4
Thickness at symphysis	14.5-19.5	4
Thickness behind M1	17-19	4

The occipitals are stoutly built. In most specimens the bone is heaped up along the course of the lambdoid suture, accentuating the obelion depression. In the six specimens where the bone is well preserved a prominent mound-shaped transverse torus is present. In two cases the torus terminates at or about the occipito-mastoid suture; in the remainder it crosses the suture and passes into a prominent occipito-mastoid ridge. In its mildest form the torus is surmounted by a suprainiac fossa, but in most cases it is separated from the occipital plane by a broad sulcus supratoralis which extends laterally to the asterion. As in most recent Australians the external occipital protuber-

ance is absent. Beneath the occipital torus the nuchal plane carries robust muscle impressions with a midline external occipital crest that passes into the base of the torus above it.

Almost all the cranial bases are badly preserved. The mastoid processes are large. Laterally medium occipito-mastoid crests are linked with distinct angular tori. The digastric fossae are wide. In two cases the juxtamastoid process is absent on one side and in one it is absent on both sides. The glenoid fossae vary with age but always large and deep. The petrotympanic fissure is associated with, and posterior to, a small postglenoid tubercle (the peculiar and unique form of the glenoid fossa in the Solo population is not seen in this series). The squamous suture is weakly to moderately arched.

The Kow Swamp faces are broad and prognathic, with clearly defined muscle attachments. The malars are large and have the following measurements: infraorbital to masseteric margins, 25–32 mm; total height, 43–52 mm; breadth of frontal process, 13–20 mm. They are thick and have everted lower borders. The malar tuberosities are expanded into thick ridges 15–20 mm long. In KS7 these ridges rise on the orbital margin at the maxillo-malar suture and sweep across the bone and upwards, almost to the fronto-malar suture. The maxillae appear rectangular from in front because the thick canine roots produce bulging of the buccal plate. The degree of subnasal prognathism is medium to large. Infraorbital fossae are present but they are always shallow. The pyriform apertures

between the central incisor roots. The groove can be distinguished from the sulcus intertoralis, at least anteriorly.

The combination of an indistinct to slight mental trigone and mild anterior incurvature produces a negative or neutral "chin". A distinct submental notch is always present, in KS7 to the extent seen in the Mauer mandible. The planum alveolare is moderately inclined. Two specimens exhibit well developed genial pits, associated in one case with vestigial genial spines. The digastric fossae are large and deep, and are clearly demarcated anteriorly and separated medially by an interdigastric ridge.

Although only one individual is of advanced age, the teeth of most specimens show pronounced attrition. Many teeth retain merely a scrap of enamel or none at all. Masticatory stress, associated with pronounced molar roll, has led to buccal exposure of the mandibular molar roots. The exposed roots of the mandibular first molars are commonly worn halfway to their apices. A maxillary incisor in KS13 is worn lingually over its total length, to such an extent that only a tiny remnant of the labial wall of the root canal is discernible. Only one dentition is complete and unworn enough for overall measurement (Table 4). The figures seem to be average for the population. All adults exhibit extensive alveolar pathologies. The chronic periodontitis, evident in many individuals, is the result of severe attrition leading to root canal exposure and inflammation due to calculus.

Table 4 Dental Measurements of Kow Swamp I (left side in mm)

Maxillary	Mesiodistal	9.0	8.1	8.1	7.25	6.75	> 10.8	11.1	12.2
	Buccolingual	8.4	7.7	8.6	9.6	10.6	> 13.6	13.8	13.4
Mandibular	Mesiodistal	5.65	5.9	7.15	7.6	8.6	11.8	12.7	13.1
	Buccolingual	6.05	6.8	8.6	9.8	8.8	> 13.0	> 12.5	12.4

are broad (27–31 mm). Their inferior borders are distinctly non-anthropine, the usual form being one where the crista prenasalis fades rapidly or curves weakly to the midline below the anterior nasal spine. Four palates have been reconstructed: all are U-shaped, with slight expansion posteriorly. They are deep (15–20 mm) and lack either buccal or lingual maxillary tori. All possess large mounded palatine tori.

By any standards the Kow Swamp mandibles are very large indeed (Table 3). All are too large to fit a cast of the Rhodesian cranium. The rami display extensive and rugose masseteric fossae, mildly developed lateral eminences and deep sigmoid notches. The gonion is markedly everted in two specimens. The condyles are narrow anteroposteriorly, compared to breadth (20–28 mm). Medially the torus triangularis is well developed. The generally shallow mylohyoid grooves are partially roofed in one specimen.

The medial surfaces of the mandibular bodies are relatively smooth, with mild development of mylohyoid ridges and submaxillary fossae. There is no evidence of a mandibular torus. The mental foramen is single and lies between PM2 and M1. In three specimens anterior and posterior marginal tubercles are well developed. In KS7 the tubercles are superimposed on a broad marginal torus or flange. This bilateral structure rises from the lateral portion of the mental trigone, sweeps sharply upwards and over the region of the anterior marginal tubercle and then flows smoothly downward to the region of M2 as a thickened, everted ridge. Above the anterior part of this torus is a groove 4 mm wide. The groove is sharply defined above by a ridge that develops at the second premolars. The groove runs horizontally for almost 20 mm before expanding, its superior margin curving upwards to meet its fellow

It is significant that the archaic features in the population are concentrated on the mandibular body and on the cranium forward of the coronal suture. Although much more recent than equivalent material from other parts of the world, the morphological complex indicates long term preservation of early *sapiens* characteristics in southern Australia. This is demonstrated by cranial size, vault bone thickness, the form of the face and mandible, and to a lesser extent the occipital region. The frontal bones are particularly archaic, preserving an almost unmodified eastern *erectus* form, specifically that of the Javan pithecanthropines.

It is likely that modern cranial form was present elsewhere in Australia some 15,000 years earlier^{2,7}, so that the relatively late dating of Kow Swamp population raises several questions. Expansion of this series of skeletons may indicate whether modern Australoid form was derived from a Kow Swamp-like source directly, or was the result of the hybridization of distinct physical types on the continent in the late Pleistocene. In either case the Kow Swamp series represents an isolated and remnant population.

Received January 17; revised May 23, 1972.

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X-ray Emission from Intergalactic Gas in the Neighbourhood of Galaxies

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Massive galaxies may have X-ray coronas associated with accretion of hot intergalactic gas. X-ray observations of Virgo A fit this hypothesis. Andromeda may also have an observable X-ray corona.

We have discussed¹ the possibility that the Local Group of galaxies might contain a low density high temperature plasma² which would be detectable in soft X-rays. In our earlier work we took into account the gravitational influence of the Galaxy on the intergalactic gas and found that in the neighbourhood of the Galaxy there could be significant enhancement of the density and temperature of the gas. In order for this enhanced gas to be responsible for the observed³ excess soft X-ray flux it would need to have an unenhanced particle density $\sim 2-5 \times 10^{-4} \text{ cm}^{-3}$ and an unenhanced temperature $\sim 2-30 \times 10^5 \text{ K}$.

Here we discuss the X-ray flux due to an enhancement around external galaxies, and to illustrate our results we choose two particular galaxies, M31 and M87, which are of special interest at present. Because M31 is in the Local Group the gas surrounding it may have the same unenhanced parameters as the gas surrounding our own Galaxy, and a successful detection of soft thermal X-rays from the neighbourhood of M31 would both provide evidence for the existence of a Local Group plasma and enable one to make estimates of its density and temperature. M87, on the other hand, is in the Virgo cluster, and it already appears to have been observed⁴ as an extended X-ray source with an angular diameter $\sim 1^\circ$. This extension could be due to X-ray emission from an enhancement of the plasma within the Virgo cluster of galaxies.

Nature of the Intracluster Gas

Although the existence of a significant intracluster gas is still uncertain, there is suggestive evidence for it from the success of the intergalactic ram pressure theory^{5,6} of double radio sources, from the X-ray emission of the Coma cluster^{7,8}, from the properties of high-velocity HI clouds in the Galaxy⁹, and from our own suggestion that part at least of the diffuse soft X-ray background may be due to an enhance-

ment of the Local Group gas surrounding our own Galaxy. Most of these considerations lead to estimates of the unenhanced particle density in the range 10^{-4} to 10^{-3} cm^{-3} . We shall take $3 \times 10^{-4} \text{ cm}^{-3}$ as a representative value for both the Local Group and the Virgo cluster, but our results for the X-ray flux simply scale as the square of the unenhanced particle density.

In order to estimate the unenhanced temperature, we follow the suggestion¹⁰ that the gas is heated by shock waves attached to galaxies moving supersonically through the gas. Such galaxies would continue heating the gas until their Mach number is reduced to about 1, that is, until the speed of sound in the gas rises to the order of the average velocity of the galaxies in the cluster. For the Local Group this average velocity is $\sim 100 \text{ km s}^{-1}$, which corresponds to a temperature $\sim 10^6 \text{ K}$. Our previous arguments¹ concerning the soft X-ray background also point to an unenhanced temperature of this order. The radial velocity dispersion of the Virgo cluster is¹¹ 700 km s^{-1} , which corresponds to a temperature of $6 \times 10^7 \text{ K}$. We note that this shock heating hypothesis is compatible with the X-ray observations⁸ of the Coma cluster. These observations imply a gas temperature $\sim 7 \times 10^7 \text{ K}$, which corresponds to a radial velocity dispersion of 750 km s^{-1} , in good agreement with the observed¹¹ dispersion ($1,000 \text{ km s}^{-1}$).

The enhancement around a galaxy is due to its gravitational action, which may even lead to an accretion of the intergalactic gas in its neighbourhood. The condition for an accretion is that the internal pressure in the galaxy is less than the external pressure enhanced by the effects of gravity. In the case of our own Galaxy it is uncertain whether this condition is satisfied, and it is possible that the Galaxy supports a static atmosphere¹² or even is the source of an outgoing wind¹³⁻¹⁵. But for a given unenhanced density and temperature the X-ray emission from a static atmosphere is at most a factor 2 greater than that associated with an accretion, so long as cooling can be neglected. It can be shown that for both M31 and M87 cooling can indeed be neglected in the temperature ranges we are considering.

Importance of Accretion

The chief question therefore is whether or not there is a wind. For our own Galaxy we tentatively inferred that there is no wind because of the observed behaviour of the high-velocity HI clouds⁹. By analogy we make the same tentative assumption for M31. For M87 we have compared the internal pressure¹⁶ with the external pressure which would be required

by our interpretation of the extended X-ray emission from this galaxy, and again conclude that accretion is occurring. We present our results for the case when each galaxy is accreting at the maximum possible rate, which actually represents a lower bound for the emission so long as there is no wind. It has been shown¹⁷ that if cooling is unimportant the enhancement due to an accretion process approximates closely to that of a stationary galaxy if the Mach number of the galaxy is less than 1.5. If the galaxy is moving subsonically the difference in the density distribution in the two cases is everywhere less than 4%, and for Mach numbers less than 1.5 it nowhere exceeds a factor of 2. Because we expect the Mach number to be of order unity, it is sufficient at this stage to consider the galaxy to be at rest relative to the gas, for which case the analytic results of Bondi¹⁸ are available. Strictly speaking, these results apply for zero magnetic field, but it can be shown¹ that magnetic effects are negligible provided that the field is much less than $2T_6^{1/2}$ microgauss where $10^6 T_6$ is the unenhanced temperature of the gas. For both galaxies we expect $T_6 \geq 1$, so this condition is likely to be amply satisfied.

Gravitational Effects

It remains to consider how best to represent the gravitational fields of the galaxies. Our previous calculations¹ showed that for our own Galaxy it is adequate to use simply a point mass at its centre. Similar considerations apply to M31, which we represent as a point mass of $2.2 \times 10^{11} M_\odot$ (ref. 19) at a distance of 690 kpc (ref. 20). We have calculated the integrated X-ray emission along lines of sight lying in various directions in the general vicinity of M31 out to 1 Mpc. In Fig. 1a we plot the photon flux at 0.5 keV as a function of angular distance θ for unenhanced temperatures in the range 3 to 18×10^5 K. We have chosen 0.5 keV as the best energy to look for this effect because at higher energies the flux is cut off due to the exponential factor in the free-free emission formula and at lower energies Galactic absorption is very severe. In order to allow for absorption the photon flux in Fig. 1a should be multiplied by a factor A where $A=0.51$ if the interstellar medium is smooth and $1 > A > 0.51$ if the medium is clumped into optically thick clouds. This result was calculated using the cross-sections of Brown and Gould²¹, a hydrogen column density in the direction of M31 of $1.1 \times 10^{21} \text{ cm}^{-2}$ (ref. 22) and a helium/hydrogen ratio of 0.1 by number. We also found that the calculated spectrum within the range 0.25 to 1.00 keV can be fitted well by an exponential spectrum with an "effective" temperature as shown in Fig. 1b.

Table 1 Parameters Defining X-ray Flux from M31

T_6	0.3	0.6	1.0	1.8
α	4.4	2.6	0.8	0.3
β	1.3	1.4	3.7	2.0

It is seen from these diagrams that the predicted flux is in the observable region unless the density of the Local Group plasma is rather low. We recognize that there may be additional sources of soft X-rays in the form of discrete objects in the Galaxy²³. Nevertheless scans across the region surrounding M31 especially at energies around 0.5 keV where the Galactic absorption is low should reveal whether there is an additional X-ray flux arising from the accretion process considered here. If such a flux is detected we could infer the unenhanced temperature of the plasma from the angular width of the emission and the unenhanced density from its absolute intensity. If we approximate the dependence of flux F on angular distance θ of our line of sight from M31 by the

relation $F(\theta)d\theta \propto \theta^{-\alpha}d\theta$, then α is a function of T_6 only. This function is shown in Table 1 for an X-ray energy of 0.5 keV. Having determined α and hence T_6 we could then calculate the unenhanced density $10^{-3} n_{-3}$ from the total flux G contained in the range of directions from 2° to 6° . This flux G is given by

$$G = \beta n_{-3}^2 A \text{ photons (cm}^2 \text{ s keV)}^{-1} \quad (1)$$

where β is a function of T_6 given by Table 1 (for an X-ray energy of 0.5 keV) and A is again a factor representing Galactic absorption.

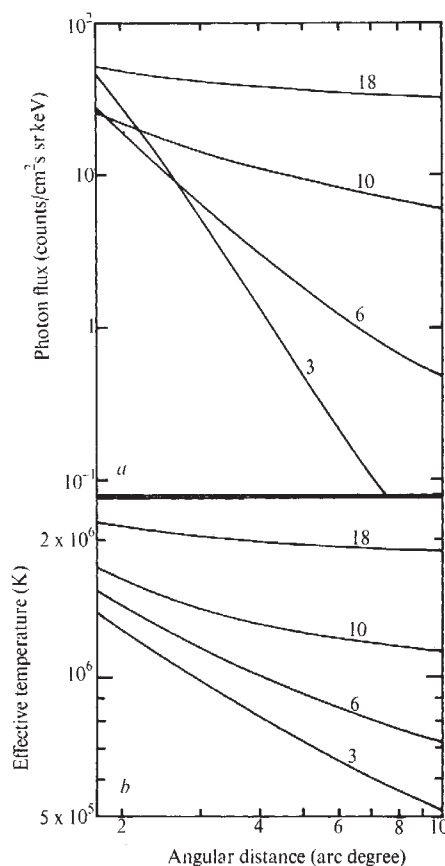


Fig. 1 X-ray flux from an enhanced region of a Local Group plasma in the vicinity of M31; the enhancement is due to the galaxy's gravitational influence. The unenhanced density of the plasma is $3 \times 10^{-4} \text{ cm}^{-3}$ and the unenhanced temperature is 3, 6, 10, 18×10^5 K according to the numbering on the curves. Diagram *a* gives the photon flux at 0.5 keV and *b* gives the effective temperature for a thermal fit to the spectrum.

In deriving a model for M87 we have used the latest observational data which show that this elliptical galaxy extends to at least 1° (refs. 24 and 25). Using the photoelectric results of de Vaucouleurs²⁴ we found that the variation of optical intensity with radius can be fitted very well by a power law. This fit is for the corona of M87 which emits most of the light. In terms of B magnitudes, the surface brightness varies as $R^{-1.6}$ where R is the radius and therefore the light density varies as

$R^{-2.6}$. The mass of M87 as given by Arp and Bertola²⁵ is $3 \times 10^{13} M_{\odot}$ which corresponds to a mass to light ratio of 200. If $M(R)$ is the mass within radius R and we assume a constant mass to light ratio then $M(R) \propto R^{0.4}$ and our model is given by

$$M(R) = \left\{ \frac{R}{R_0} \right\}^{0.4} M_{\odot} \text{ for } 0 < R < R_0$$

$$M(R) = M_0 \text{ for } R > R_0$$

Here R_0 is the limit out to which de Vaucouleurs' observations extend (26 arc min or 125 kpc, taking the distance of M87 to be 16.6 Mpc²⁶) and M_0 is $3 \times 10^{13} M_{\odot}$.

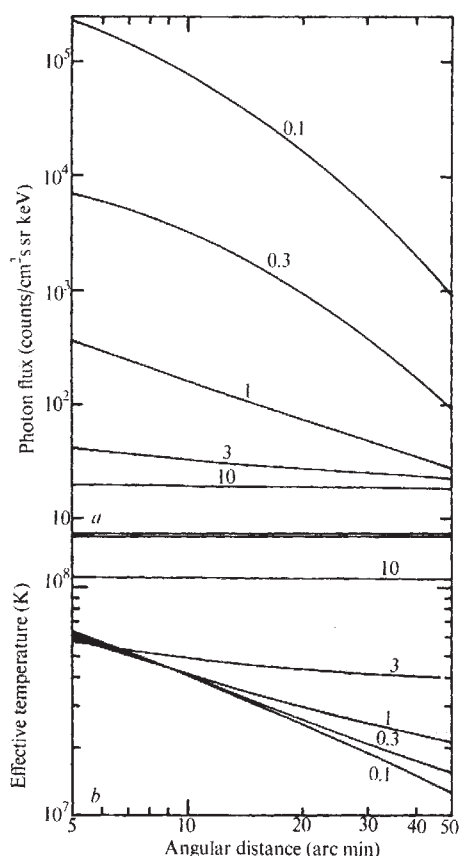


Fig. 2 X-ray flux from an enhanced region of a plasma within the Virgo cluster in the vicinity of M87. The unenhanced density is $3 \times 10^{-4} \text{ cm}^{-3}$ and the unenhanced temperature is 0.1, 0.3, 1, 3, $10 \times 10^7 \text{ K}$ as labelled on the 5 curves. Diagram *a* gives the photon flux at 4 keV and *b* gives the effective temperature of the associated thermal spectrum.

With this model we can no longer use the results for a point source and we need to modify Bondi's analysis so that it applies to an extended source. With an extended source we expect material to be absorbed within the region $R < R_0$. This means that for $R > R_0$ the accretion rate λ is constant but for $R < R_0$ λ is a function of R . The variation of λ with R is unknown but if we choose λ so that it is a maximum for each R then the density of the flow, and hence the resulting emission,

is a minimum. The results shown in Fig. 2 are derived using this accretion rate and represent a lower bound to the emission for a given unenhanced density and temperature. The upper bound occurs when $\lambda = 0$ (that is, for a static atmosphere) for which the emission is at most a factor 3 above the minimum value. If material is not absorbed in the corona of M87 but in its nucleus then the accretion rate is small (about 10% of maximum) and the fluxes shown in Fig. 2 should be multiplied by ~ 2 .

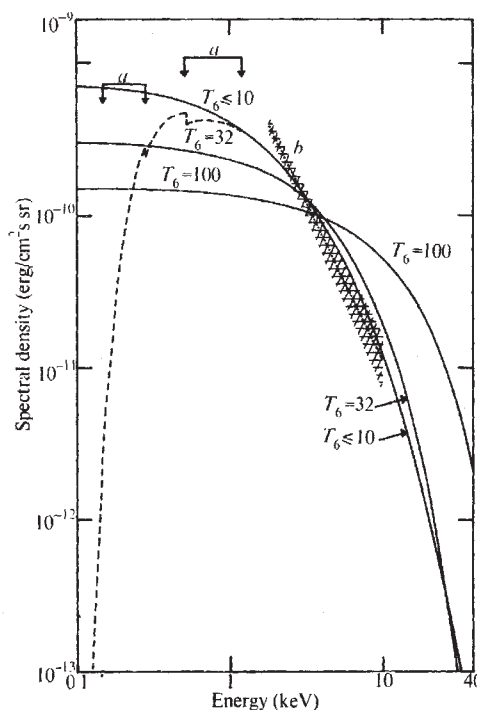


Fig. 3 The spectrum from an enhanced region around M87 integrated over angular radius 5 to 50 arc min as a function of T_6 . The density n_{-3} is chosen such that the curves are a best fit to the UHURU data²⁹ shown by the dotted line *b* (Kellogg *et al.*²⁹), the hatched area being the 1σ error box. The dashed curve is the spectrum when Galactic absorption due to interstellar hydrogen is taken into account for $T_6 \leq 10$. The column density is $3 \times 10^{20} \text{ cm}^{-2}$ (ref. 28) and the absorption coefficient is taken from Brown and Gould²¹. The soft X-ray limits of Gorenstein *et al.*²⁸ are also shown (*a*).

In Fig. 2*a* we have plotted the expected photon flux at 4 keV as a function of angular distance for initial temperatures of the plasma in the range 10^6 to 10^8 K with initial density of $3 \times 10^{-4} \text{ cm}^{-3}$ to facilitate a direct comparison with the results for M31. The emission was integrated over a path length of 2.5 Mpc, corresponding to the diameter^{11,26} of the Virgo cluster. As with M31 the emission can be well represented by an exponential spectrum, in this case in the energy range 0.25 to 16 keV. The dependence of this effective temperature on angular distance is given in Fig. 2*b*.

We can also deduce from a positive X-ray observation both the initial temperature and density of the gas. If we represent the observed flux at 4 keV in directions θ between 5 and 50 arc min by a power law $F \propto \theta^{-\alpha}$, we determine the unenhanced temperature T_6 from the values of α given in Table 2. We can then determine the unenhanced density n_{-3} from the total observed flux G at 4 keV between 5 and 50 arc min using equation (1) with β now determined in terms of T_6 by Table 2.

The attenuation factor A is set equal to one because there is negligible absorption of 4 keV photons by the interstellar medium. From the present observational data we can set stringent limits on the density of the gas within the Virgo cluster. Using the data from UHURU⁴ we can consider the emission as coming from an extended region of angular diameter 1.3° having a flux of 1.8×10^{-10} erg cm⁻² s⁻¹ in the range 2.4 to 6.9 keV. This gives $G \sim 10^{-2}$ and the corresponding values of n_{-3} are shown in Table 2. The observations of

Table 2 Parameters Defining X-Ray Flux from M87

T_6	1	3	10	30	100
α	2.4	1.9	1.1	0.2	0.0
β	85	3.9	0.39	0.19	0.14
n_{-3}	0.011	0.051	0.16	0.23	0.26

Lampton *et al.*²⁷ in the range 1 to 10 keV give a similar result for G . If the UHURU data are fitted with a thermal spectrum the effective temperature must be greater than 2.5×10^7 K which implies $T_6 > 5$. From Table 2 it is apparent that within the range 0.5 to 10×10^7 K the density cannot be more than $1-3 \times 10^{-4}$ cm⁻³. This result depends on our having adopted Arp and Bertola's²⁵ rather large mass for M87. But taking a conventional mass to light ratio of 50 for an elliptical galaxy would give the mass as $1.2 \times 10^{13} M_\odot$ which only alters the density range slightly, to $2-3 \times 10^{-4}$ cm⁻³. The upper limits of the soft X-ray flux from Virgo²⁸ also place upper limits on the value of n_{-3} but these limits are everywhere less severe than from the results at 4 keV.

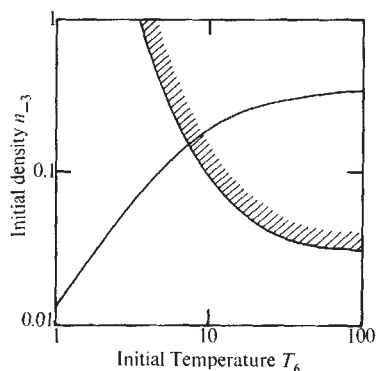


Fig. 4 The initial density n_{-3} of the gas such that the total flux is that observed by Kellogg *et al.*²⁹ (see Fig. 3). The hatched curve is the limit imposed by the observational constraint that less than 4×10^{-9} erg (cm² s sr)⁻¹ comes from the Virgo cluster itself in the range 2-6 keV. This requires that $T_6 < 7$ and $n_{-3} < 0.15$.

In order that the proposed accretion process may be unscrambled from the other X-ray processes occurring in M87 and that the value of α may be determined, it will be necessary to scan M87 with at least 0.1° resolution which is perhaps too hopeful at present. But this does not apply to M31 where a 1° resolution would be adequate to determine both α and β and hence the density and temperature of the Local Group plasma. A null result would place stringent limits on the density of the plasma and therefore we suggest that this effect be looked for particularly in the neighbourhood of M31 and other similar galaxies.

We thank T. B. Austin for his help in deriving a model for M87 and K. A. Pounds for a helpful discussion.

Addendum

Shortly after submitting this article we received a preprint²⁹ which reports the most recent UHURU data on M87. These

data indicate that at least 65% of the flux comes from an extended region centred on M87 and of diameter at least 50 arc min., with no detectable long-term variations. (The remaining 35% of the flux may come from an unresolved source.) A previous suggestion⁴ that some of the flux comes from the neighbouring galaxy M84 is now withdrawn. In order to compare our predictions with these new measurements, which refer to the extended source as a whole, we have integrated the calculated flux shown in Fig. 2 over the range of directions from 5 to 50 arc min in angular radius from M87. The results are shown in Fig. 3. For $T_6 \leq 10$ the integrated spectrum is independent of T_6 to a precision of a few per cent and agrees with the UHURU spectrum and the upper limits of Gorenstein *et al.*²⁸ for a suitable choice of n_{-3} . The required relation between n_{-3} and T_6 is shown in Fig. 4. This figure also shows the region of the n_{-3} , T_6 plane that is forbidden by the UHURU upper limit of 4×10^{-9} erg (cm² s sr)⁻¹ for the general background flux in the range 2 to 6 keV from the Virgo cluster. The two conditions taken together require that $T_6 < 7$ and $n_{-3} < 0.15$. In addition we note that for $T_6 > 30$ the predicted spectrum of M87 differs substantially from the observed spectrum. Finally we note that all the known³⁰ extended extragalactic X-ray sources are located in rich clusters of galaxies, where the accretion process may be occurring¹⁰ provided that the peculiar velocity of the galaxy is not too large (a condition which may be satisfied, for example, by NGC 1275 (ref. 31)).

Received March 30, 1972.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Mars: Are Observed White Clouds composed of H₂O?

MARTIAN clouds have been classified¹ into Type I, which are visible at short optical wavelengths, and become gradually invisible as the wavelength increases; and Type II, which are visible at long wavelengths, and fade into invisibility when the wavelength decreases. The three principal kinds of observed Martian clouds—yellow, white, and blue—can be categorized as Type I, blue; and Type II, yellow and white. This distinction is essential. Yellow and white clouds are classified together because they are visible in the same wavelength range; yellow clouds are dust clouds and will not be described here.

The composition of white and blue clouds is uncertain, although their physical distinction has been evident for some time² (Fig. 1). The positive polarization branch for blue clouds between phase angles 0° and 20° implies that they are made up of much smaller particles than white clouds, whose polarization over the same phase angle range is negative. This property explains the separate spectral categorization: smaller particles scatter shorter wavelengths more efficiently than larger particles. I have revised my lists of white cloud occurrences that have been observed on Mars for the past century³ by measuring the areographic coordinates of the clouds listed in Table IIb of ref. 3 on photographic plates of Mars, and examining the instances of clouds which have been measured polarimetrically.

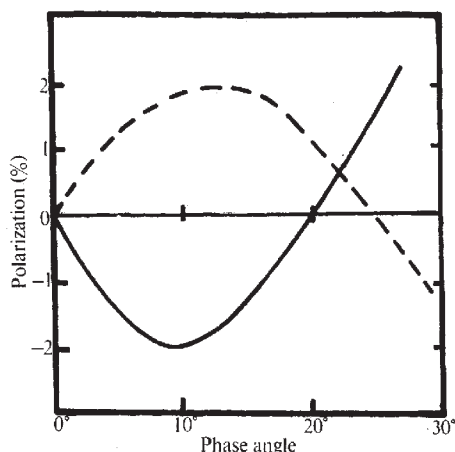


Fig. 1 ---, Polarization of Martian blue clouds; —, polarization of Martian white clouds according to Dollfus². Compare the branches between phase angles 0° and 20°.

The value of polarization as a function of phase angle provides a nearly unambiguous means of distinguishing between white, blue, or yellow clouds, thin atmospheric veils, and surface frost deposits. Yellow and white clouds and surface frost have similar appearances on black and white photographic plates, and in the absence of polarization data supplementary information must be used to help distinguish the type of phenomenon. For example, does the phenomenon occur on the

limb of the planet; what is the planetocentric location of the occurrence; the season of the Martian year; and the telescopic colour appearance of the phenomenon (if available) at the time the photograph was made, compared with the brightness of the polar cap?

More complete details of Martian clouds and their detection, including the new lists, will be published later.

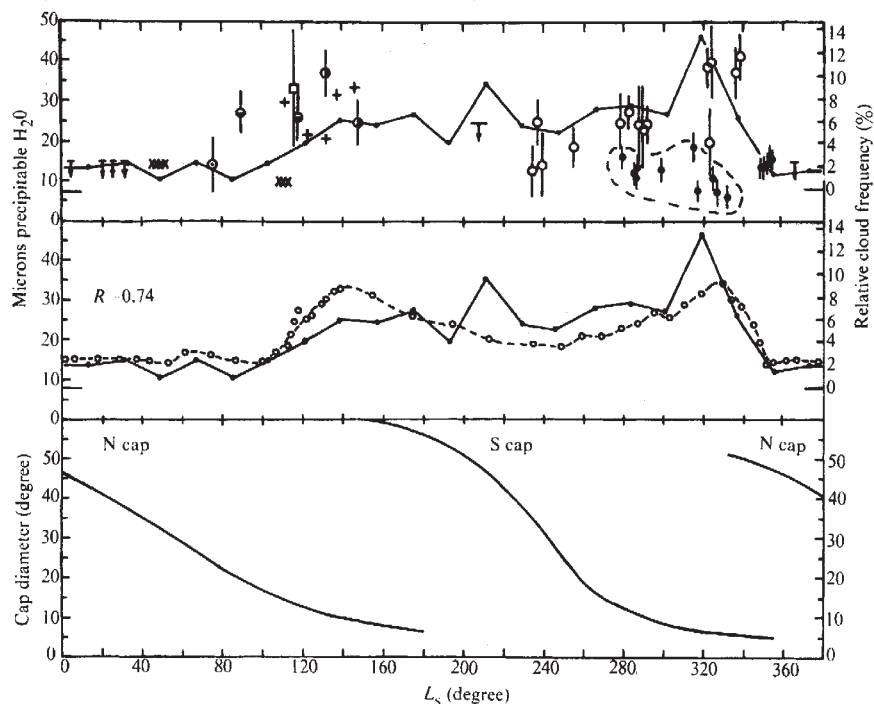
The revised list of white clouds contains 252 occurrences which are divided as follows: telescopic reports in the literature in which colour distinction and identification as a cloud rely solely on the original observer (1858–1941), 27%; photographic plates in which I made identifications according to the guidelines established previously (1937–1963), 40%; and identification by polarimetric methods (1954–1971), 33%. The percentage categories are listed according to increasing accuracy of identification as white clouds. In those instances where the years of observation overlap the different methods, some clouds were identified by more than one method. This provided a valuable cross-check on the reliability of the less accurate means of identification.

I previously made a comparison⁴ between the latitudinal distribution of white cloud occurrences on Mars and the early water vapour data of Schorn *et al.*⁵ In the region of the Martian orbit where their spectra overlapped the cloud distributions, the frequency of white clouds in the northern hemisphere was between three and four times larger than in the southern hemisphere. At the same time, water vapour was more prevalent in the northern hemisphere, indicating that Martian white clouds could be composed of water. But the spectral H₂O data were not very extensive in seasonal coverage so that agreement between white cloud frequencies and the abundance of water vapour could have been fortuitous. Since then, Barker *et al.*⁶ have summarized all available H₂O data up to 1969 in the form of a seasonal coverage chart. These data have been revised and extended to 1971 by Tull⁷.

I have made a direct comparison of these data with observed white cloud frequencies (Fig. 2) by plotting H₂O abundances and white cloud frequencies against L_s , the planetocentric longitude of the Sun (the heliocentric orbital longitude of Mars, η , and the planetocentric longitude of the Sun are approximately related by $\eta = L_s + 85^\circ$). The 252 clouds were counted in 18° intervals of heliocentric longitude, and the points of the frequency polygon were plotted on the interval mid-points in terms of L_s . On the right ordinate, the relative cloud frequency scale has been adjusted by prior sliding of overlay plots so that the variations in cloud frequency and H₂O vapour can be seen to best advantage.

The top diagram shows the cloud frequencies compared with the original H₂O measurements. The filled circles in the range $280^\circ < L_s < 360^\circ$ are recent observations⁷ from the 1971 Mars apparition. Those points enclosed within the dashed-line envelope represent measurements made between October 1971 and January 1972 during the maximum activity of the yellow dust storm which enveloped the planet during this period. These observations reflect only the H₂O vapour above the yellow clouds⁷. Observations of global CO₂ pressures⁸ and the Mariner 9 photography⁹ during the same period indicate that the tops of the yellow clouds are at an altitude of either 10 km or 40 km depending on whether a single- or multiple-scattering layer model is used.

Fig. 2 Global white cloud frequency distribution based on 252 occurrences around the Martian orbit compared with the observed abundance of water vapour as a function of season on Mars. In the upper diagram, vertical arrows point downwards from upper limits, Schorn *et al.*⁵; $\times$, $\odot$, Schorn *et al.*⁵; $\square$, Owen¹⁹; $+$, $\bullet$, Tull¹⁶; $\circ$, Kaplan *et al.*²⁰; $\circ$, Barker *et al.*⁶; $\bullet$, Tull and Barker⁷. In the middle diagram, the cloud frequency ($\bullet$ — $\bullet$) is compared with a 5-point running average of the H_2O data ($\circ$ — $\circ$) in the upper diagram, omitting the points enclosed by the dashed envelope. The lower diagram shows the polar caps diminishing with season as determined by Antoniadi¹⁰.



The other five points between about $L_s = 350^\circ$ – 360° refer to observations made towards the end of February and the beginning of March 1972 after the dust storm had subsided.

The middle diagram shows the distribution of cloud frequencies compared with a five-point running average of the H_2O data points in the upper diagram. The ten points enclosed by the envelope in the top diagram were omitted from this averaging process because of the influence of the dust storm.

Table 1 Rank Correlations and Significance Levels for Figs. 2 and 3

Significance level	Normal z deviate	Spearman's R	No. ranks	s.e.	Calc. z deviate	Null hypothesis rejected
0.200	0.67					
0.100	1.65					
0.050	1.96	0.74	20	0.229	3.23	Yes
0.020	2.33	0.56	7	0.408	1.37	No
0.010	2.58	0.94	6	0.447	2.10	Yes
0.001	3.29					

A peak in cloud frequency of 9.5% at $L_s = 211^\circ$ appears to be out of place. It would have been desirable to have had more H_2O data between $L_s = 150^\circ$ – 230° , but it is clear that the variations in cloud frequencies are correlated with those of the H_2O abundances. For if intervals of cloud frequency are ranked by increasing frequency with the corresponding increases in H_2O abundances, Spearman's rank correlation gives $R = 0.74$.

An exact test for the level of significance of the rank correlation coefficient is laborious since there are 20 ranks; however, if the standard error of R is approximated by $(n-1)^{-1/2} = 0.229$ for the n ranks, then the normal z deviate is $0.74/0.229 = 3.23$. The significance test rejects the null hypothesis because at the 1% level $z = 2.58$ and at the 0.1% level $z = 3.29$ (Table 1). I therefore conclude that the correlation between white cloud frequencies and H_2O abundances is significant.

The bottom diagram of Fig. 2 shows the decreasing size of the Martian north and south polar caps with season¹⁰. Clearly more H_2O and white clouds occur in the atmosphere when the polar cap size is at a minimum. It is known from observation^{11,12} and theory^{13,14} that the Martian polar caps are primarily frozen

CO_2 , although theory admits a two-layer model composed of both frozen CO_2 and H_2O ice. Also, recent measurements¹⁵ from Mariner 9 have indicated the presence of H_2O vapour over the south polar area.

It is interesting to compare H_2O abundances with those of white clouds as a function of latitude on Mars. Tull¹⁶ has published H_2O abundances distributed by latitude and measured on 2 days separated by a month in March and April 1969. During this period, Mars ranged from about $\eta 218^\circ$ to 231° heliocentric longitude. Two 18° intervals contain a statistically significant cloud count corresponding closely to these dates, $\eta 216^\circ$ to 233° and $\eta 234^\circ$ to 251° .

In these intervals 43 clouds occurred and are divided as follows: telescopic reports in classical literature, 12%; identification by photographs, 39%; and identification by polarization, 49%. Clouds were counted in 10° latitude intervals (Fig. 3).

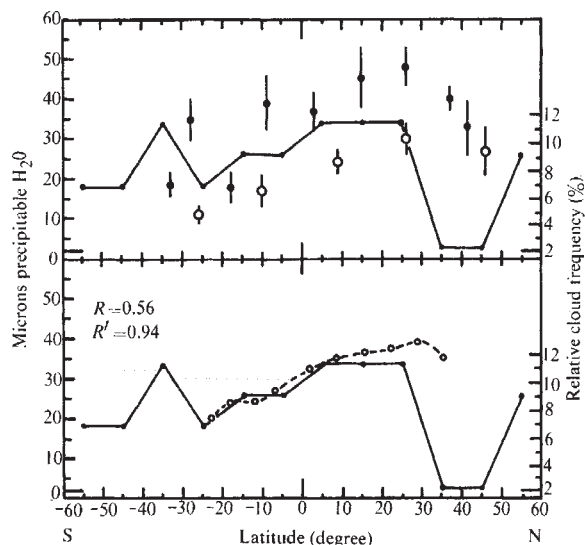


Fig. 3 $\bullet$ — $\bullet$, White cloud frequencies distributed by latitude for the portion of the Martian orbit between $\eta 216^\circ$ – 251° compared with the H_2O abundances determined by Tull¹⁶. In the upper diagram, observations for March 27 are denoted by $\bullet$ and for April 28 by $\circ$. In the lower diagram, the cloud frequency ($\bullet$ — $\bullet$) is compared with a 5-point running average ($\circ$ — $\circ$) of the H_2O data in the upper diagram.

The filled circles in the upper diagram are Tull's H₂O abundances for March 27, the open circles for April 28. In the lower diagram a 5-point running average has been applied to the H₂O data. Over the portion of the diagram from about -30° S to about 25° N, the correspondence between the cloud frequencies and H₂O abundances is quite good.

In the lower diagram, there are 7 frequencies between -25° S and 35° N which have matching H₂O data. But for these 7 ranks Spearman's correlation coefficient is only 0.56. For less than 10 ranks a significance test may not be valid; however, line 2 of Table 1 gives the standard error and calculated *z* deviate. The calculated value, 1.37, does not exceed that expected for the 10% significance level; therefore, the null hypothesis cannot be adequately rejected. On the other hand, if only the 6 points between ±25° latitude are ranked with the corresponding water vapour data, *R'* = 0.94. In that case the calculated *z* deviate, 2.1, exceeds the 5% level and is close to the 2% level; hence, this correlation is probably significant (Table 1).

It is not necessarily the case that observed cloud frequencies will always follow H₂O abundances. In Fig. 2, for example, the trends are averaged over an entire Martian year; in Fig. 3, the trend is a monthly average. Many atmospheric conditions will affect the rates of condensation and/or sublimation and thus cloud formation. In particular, the severe drop in cloud frequency between latitudes 35° and 45° N poses an interesting problem. It suggests that perhaps some atmospheric process is suppressing cloud formation, other factors being equal. But an equally vexing problem is posed by the apparent rise in cloud frequency in the opposite hemisphere at -35° S latitude, if indeed this is real. Over this range of the Martian orbit, the northern hemisphere is experiencing summer, while the southern hemisphere is in winter. This fact may affect the vertical temperature profiles and hence the H₂O condensation rates which could explain the cloud frequency peak in the one hemisphere and the minimum in the other.

Although Martian white cloud frequencies tend to follow the distribution of H₂O both seasonally and latitudinally on the planet, which strongly suggests that most white clouds are formed from water vapour, evidence for the composition of the blue clouds is still lacking.

Polarization data (Fig. 1) indicated that blue clouds are composed of much smaller particles than white clouds. This distinction implies either a different composition for blue clouds or a different physical environment in which they form, or both. The observation by Herr and Pimentel¹⁷ of solid CO₂ crystals above the planetary limb during the flights of Mariners 6 and 7 and the meteorological analysis of this observation by Hess¹⁸ indicate the possibility of two layers of condensation on Mars. Hess¹⁸ pointed out that if CO₂ condensation takes place in the upper atmosphere of Mars, then H₂O condensation at lower levels is almost a certainty. So it is possible therefore that Martian blue clouds are composed of CO₂ ice.

I thank R. G. Tull for an advanced copy of the Martian H₂O observations conducted at MacDonald Observatory and the seasonal coverage chart, and A. Dollfus for permitting examination of the detailed collection of polarization measurements and the photographic plates at the IAU Mars Data Centre, Observatoire de Paris, Meudon. This investigation was supported by a NASA grant.

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Received March 21, 1971; revised May 15, 1972.

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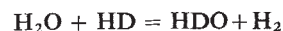
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The D/H Ratio in Jupiter's Atmosphere

DEUTERIUM has recently been detected in the methane component of the Jovian atmosphere¹. Here we estimate this ratio theoretically with the help of recent work by Geiss and Reeves².

We assume that water vapour and hydrogen gas in the proto-solar nebula are in isotopic equilibrium. Because of the small atomic abundance of deuterium relative to hydrogen, the D/H ratio for water divided by that for hydrogen gas is almost equal to the equilibrium constant for the exchange reaction:



$$K_1 = \frac{(\text{HDO})}{(\text{H}_2\text{O})} \bigg/ \frac{(\text{HD})}{(\text{H}_2)} \simeq (\text{D/H})_{\text{water}}/(\text{D/H})_{\text{hydrogen}} = \alpha_1$$

Values for *K*₁ have been reported by Bottinga³ for temperatures above 273 K. We have extended these calculations to 100 K using the same analysis. We assume that para and orthohydrogen are not in equilibrium but occur according to their normal terrestrial distribution; *K*₁ depends only on temperature. General studies of the chemical equilibrium during the formation of the solar system (ref. 4, see also E. Anders, Nobel Symposium 21) give the temperature at which these exchange reactions occur.

Because the D/H ratios on Earth and in carbonaceous chondrites are similar⁵, it is reasonable to estimate this temperature from meteorite studies; a recent analysis of the ¹²C/¹³C fractionation pattern between wax and CO₂ in carbonaceous chondrites⁶ indicates a temperature in the range 330 K to 380 K.

The difference in the relative D/H ratios for water vapour and ice⁷, and for liquid water and hydrated silicates⁸, is small enough, when these phases are in isotopic equilibrium, to be negligible for our purpose.

In the temperature interval of interest, values of *K*₁ range roughly from 3 to 4 (Table 1). Ocean water D/H abundance is about 157 p.p.m. (ref. 9) and hydrogen gas in isotopic equilibrium with such water will have a D/H ratio of (3.0 ± 1.0) × 10⁻⁵ at temperatures likely to occur in the proto-solar nebula. This ratio agrees with the upper limit of D/H ≤ 7 × 10⁻⁵ in the interstellar gas deduced by Weinreb¹⁰, with the solar wind measurement of ³He/⁴He by Geiss et al.¹¹, taking into account the (D + P → ³He) reaction during the Hayashi phase of the Sun^{2,12}.

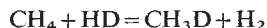
To estimate the D/H ratio for methane in the Jovian atmosphere we need to know the fractionation factor.

Again, because of the small atomic abundance of D with respect to H, we neglect molecular species such as CH₂D₂, CD₄ and D₂ obtaining:

$$\alpha_2 = (\text{D/H})_{\text{methane}}/(\text{D/H})_{\text{hydrogen}}$$

$$\alpha_2 = \frac{(\text{CH}_3\text{D})}{4(\text{CH}_4) + 3(\text{CH}_3\text{D})} \bigg/ \frac{(\text{HD})}{2(\text{H}_2) + (\text{HD})} \simeq 1/2 K_2$$

where K_2 is the equilibrium constant for the isotopic exchange reaction



We have extended Bottinga's^{1,3} calculations of K_2 to 100 K. An analysis of the observational data of Beer *et al.*¹ has given a D/H ratio of $3\text{--}7 \times 10^{-5}$ for the methane phase of Jupiter (Black, private communication). Because $K_2 > 1$ at all temperatures the value 7×10^{-5} is an upper limit to the D/H in the hydrogen phase. Actual determination of the temperature at which the equilibrium is achieved is very difficult in view of the possible convective motions in the atmosphere. Comparison with the estimate of the protosolar D/H by Geiss and Reeves² suggests temperatures of 250 K and 400 K.

Table 1 Equilibrium Constant of D/H in Water and in Methane (Data from Refs. 4–6)

T (K)	K_1	$K_2/2$
100	62.0	85.0
110	42.9	56.2
120	31.4	39.5
130	24.0	29.2
140	19.0	22.5
150	15.4	17.9
160	12.9	14.6
170	11.0	12.2
180	9.48	10.4
190	8.32	8.97
200	7.40	7.87
210	6.64	6.98
220	6.02	6.26
230	5.51	5.67
240	5.07	5.17
250	4.70	4.75
260	4.38	4.40
270	4.10	4.09
280	3.86	3.82
290	3.65	3.60
300	3.46	3.39
333	2.86	2.97
373	2.44	2.56
473	1.86	1.97
573	1.56	1.68
673	1.40	1.49

We thank a number of colleagues for discussions.

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Received March 15; revised July 3, 1972.

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Distribution of Heavy Metals in the Vicinity of an Industrial Complex

ABNORMAL concentrations of lead, cadmium and zinc exist in the head waters and sediments of the Bristol Channel, and these metals may be dispersed from the industrial complex at Avonmouth^{1,2}. Butterworth, Lester and Nickless¹ have shown also that some living organisms from these waters contain exceptionally high concentrations of the same metals. It is logical to reason that airborne pollution might originate from the same sources and the results reported here show the extent to which this hypothesis has so far been shown to be valid.

Goodman and Roberts³ have described an extensive survey of a similar problem in the Swansea district and in our investigations we have used similar criteria as evidence of airborne pollution.

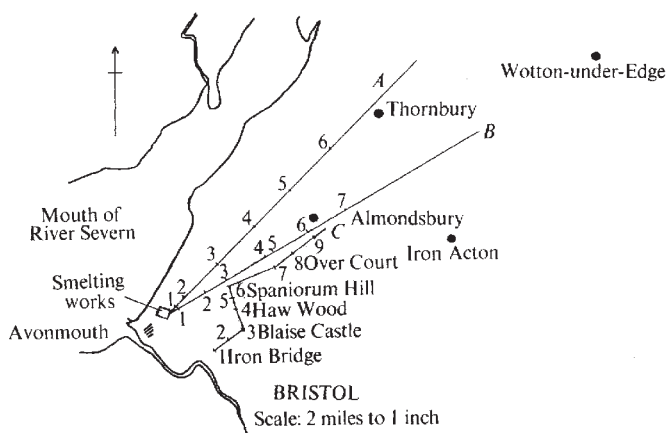


Fig. 1

Samples of grass—*Lolium perenne*; moss—*Eurhynchium praelongum*; and lichen—*Parmelia*, were collected from the sites shown in Fig. 1 and analysed for lead, zinc and cadmium. Soil was also collected. In this case a small hole was made, and the first 5 cm of earth collected and intimately mixed. Our sampling points lie along three well defined transects.

A, Bearing 46° T from the smelting works at Avonmouth.

B, Bearing 60° T from Avonmouth.

Both A and B lie across land that is used predominantly for agricultural purposes and along these transects grass and soil samples were taken from pasture land at least 50 m from the nearest road or hedge.

C, A line following the crests of a series of low hills (200–300 feet) just to the north of the main urban complex of Bristol and extending from Kings Weston to Almondsbury. From these crests there is uninterrupted low ground to the industrial complex and Channel beyond.

The samples along A and B were collected in November 1970 and again in September 1971. Those along C were collected in September 1971. The prevailing wind in the area is W–SW.

Plant specimens were carefully separated from soil or other extraneous matter and dried at 105°C for 24 h. The grasses were not washed before analysis so very small particles of soil of 10μ diameter or smaller would still adhere to the sample. To avoid metal contamination from the air or from rain splashing, moss and lichen samples were taken from 2 to 3 m above ground level. The moss was carefully sorted into species since the metal-retaining capacity of mosses varies from species to species. Weighed samples, about 1 g for grasses and mosses and 0.3 g for lichens, were digested in 10 ml. of hot concentrated 'AnalaR' nitric acid (70°C), filtered through a No. 4 sintered glass filter to remove insoluble silica material, made up to 25 ml. with double distilled water and examined for cadmium, lead and zinc with a 'Varian Techtron AA-5' atomic absorption spectrophotometer.

Table 1 Concentrations of Heavy Metals along Transect A *

Sample point	Distance from smelting works (miles)	Grass <i>Lolium perenne</i>			Soil		
		Pb	Zn	Cd	Pb	Zn	Cd
1	0.2	225	1,600	50	600	5,000	32
2	0.7	204	300	15.2	580	1,400	10
3	2.8	86	350	9	270	450	7.1
4	4.3	75	86	15	180	250	3.1
5	5.9	48	55	6.7	100	150	3.0
6	7.9	23	60	3.0	60	90	1.1

* Metal levels are given in p.p.m. in Tables 1-4.

Table 2 Concentrations of Heavy Metals along Transect B

Sample point	Distance from smelting works (miles)	Grass <i>Lolium perenne</i>			Soil		
		Pb	Zn	Cd	Pb	Zn	Cd
1	0.2	225	1,600	50	600	5,000	32
2	1.2	181	350	10.8	540	1,540	12
3	2.6	160	270	9.0	230	416	7.7
4	3.8	100	249	7.8	330	500	9.8
5	4.3	47	85	5.2	192	292	3.6
6	5.5	55	76	3.9	334	216	3.7
7	7.0	25	70	1.8	80	130	2.0

Table 3 Concentrations of Heavy Metals along Transect C

Sample point	Bearing from smelting works (°T)	Distance from smelting works (miles)	Pb	Moss* Zn	Cd	Pb	Lichen† Zn	Cd	Pb	Grass‡ Zn	Cd
1	122	2.4	100	839	86	80	531	72	9	68	6
2	112	2.5	120	1,213	93	130	675	68	10	102	8
3	107	3.0	131	1,315	122	146	832	73	26	123	8
4	86	2.6	1,200	4,870	137	1,528	1,135	83	49	146	13
5	78	2.7	1,411	6,315	140	1,631	1,839	84	112	287	14
6	74	2.9	1,623	6,835	148	1,623	3,271	90	148	338	14
7	72	4.5	864	1,839	110	631	1,261	65	41	140	13
8	67	5.2	783	1,753	97	523	671	41	37	86	10
9	64	6.0	389	876	49	417	386	20	38	78	4

* Moss: *Eurhynchium praelongum*. † Lichen: *Parmelia*. ‡ Grass: *Lolium perenne*.

Soils were dried at 105° C, ground to pass a 30 mesh BSS sieve and extracted by heating 1 g samples with 8 ml. of concentrated 'AnalaR' nitric acid. After 1 h the extract was cooled, diluted with a little water, and filtered (Whatman No. 42 paper) into a 25 ml. graduated flask. The washings, 2 × 5 ml. of double distilled water, were likewise decanted through the same filter paper before making up to the mark. Pb, Zn and Cd were then determined with the 'Varian AA-5'.

Table 4 Concentration of Heavy Metals found on Coombe Hill*, Wotton-under-Edge (Southern Cotswolds)

Grass†			Soil		
Pb	Zn	Cd	Pb	Zn	Cd
4.0	40	0.2	14	40	0.5

* Coombe Hill is on the Southern Cotswolds, bearing 59° T and 19.5 miles from the smelting works.

† Grass: *Lolium perenne*.

While this method does not give an absolute value for soil owing to some metals being still trapped in the insoluble materials, experiments with an HF dissolution process have shown that the trend is still the same although values are about 10% less than absolute. It was decided, however, to carry

out soil analyses by the digestion method so that the laboratory would have a standard simple method for all soils.

Each value is the mean of 3 values per site. We found that although the variation was small for grasses, mosses, and lichens, approximately 8%, it was larger for soils, up to 18%. This reflects the difficulties in obtaining a uniform sample from a site.

We are not completely satisfied with the sample collection methods of ourselves or earlier workers since we are not convinced that a fully representative sample can be obtained. We are examining this problem and it is hoped to publish this work at a later stage.

Our results demonstrate that very high concentrations of zinc, cadmium and lead are found near the smelting works along both the A and B transects. Although there is a rapid fall in concentration as distance from the works increases, significant quantities can still be detected seven miles away. These transects both lie within the zone of the prevailing winds.

The figures obtained along line C provide strong supporting evidence for the supposition that this is a case of airborne pollution. At point 1, to the south-east of Avonmouth and not along the line of the prevailing wind, relatively low concentrations were found, despite being closer to the centre of the urban complex than any other sampling point. Points 2, 3, etc., gradually move the sampling point away from the smelting works and away from the built-up area but nevertheless the

metal concentrations increase to a maximum at Spaniorum Hill (point 6) which is in open country. We conclude that this increase occurs as the sampling line moves into the zone of the prevailing wind from Avonmouth with the result that a greater weight of airborne contamination reaches these points. The M4 and M5 motorways probably lead to some distortion of the lead concentrations, but we feel that this distortion is small since the distribution of lead is similar to that for zinc and cadmium.

We thank Mr D. Roberts and Mr R. Stenner for help and advice. We also thank the SRC for the award of an advanced studentship to one of us (A. B.).

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Received March 17; revised May 28, 1972.

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Heavy Metal Content of Some Rivers and Lakes in Wales

WELSH rivers and lakes are remarkably free from domestic and industrial pollutants, but because of the presence of mineral deposits and the remains of mining activities, they are likely to have small but significant concentrations of heavy metals that may cause severe deterioration of the water quality. For example, the presence of lead, zinc and copper in some Welsh streams causes a high mortality rate in fish and other living populations of these streams¹⁻⁵, and the heavy metals carried to the sea by Welsh rivers are the primary source of these metals in Cardigan Bay⁶. But since the cessation of mining activities in the early part of this century, it has been reported that some rivers have shown some degree of recovery from the effects of lead pollution^{7,8}. Only limited information is available concerning the heavy metals in Welsh rivers, and most of this is not entirely reliable as an indicator of the present level of metals in the water. Here we report an investigation of the heavy metal content of rivers and lakes in Wales carried out from September 1970 to October 1971. The River Dee and its tributaries were examined between July 1968 and April 1969. Samples were taken from the positions shown in Fig. 1 at monthly intervals. Seven trace metals and the major ions were determined. The trace metals were separated by chelating resins and analysed using pulse polarography⁹ or atomic absorption spectrophotometry.

The variation in any particular stream from month to month is considerable, being on average within 60% of the mean value for the period of study; for example, the zinc content of River Twymyn water (upstream) varies between 300 and 600 µg/l. (mean, 437 µg Zn/l.) while the zinc content of the water from the upper reaches of River Rheidol varies between 40 and 82 µg/l. (mean 51 µg Zn/l.) over the same period.

Since little or no industrial waste is discharged into these streams, the heavy metals must be leached from the top soil in the mineralized zone of the region or from old mines or mining waste. The composition of uncontaminated water is difficult to estimate since this will depend to a great extent on the geological character of the watershed area. In the Welsh rivers, the minimum concentration of each metal found will be assumed to indicate the level when there is no mineral influence in the watershed area. In a "clean" stream water the concentrations of metals in $\mu\text{g/l.}$ are: Mn, 0.80; Fe, 1.7; Cu, 0.66; Pb, 0.70; Cd, 0.41; Ni, 0.50; and Zn, 11.0.

The amount of iron in stream water seems to depend on the presence of soluble organic matter such as humic material, which may stabilize the colloidal iron species to remain in "solution". The stabilizing effect of the soluble organic matter does not appear to extend to manganese, which tends to decrease rapidly downstream owing to hydrolysis and precipitation.

Different metals are found to occur at high concentrations in the various rivers. Thus, the River Ystwyth, reported in 1936 to be lead and zinc polluted⁵, and more recently to be recovering¹⁰, contains moderately high zinc and lead ranging between 200–270 µg Zn/l. and 2–6 µg Pb/l. Manganese and cadmium are moderate (7–15 and 1.1–1.3 µg/l. respectively). The River Rheidol is found to be rich in manganese (9–28 µg/l.) and zinc, cadmium and lead levels are moderately high (50–130, 1.0–3.4, 1.3–2.4 µg/l., respectively). The zinc and cadmium appear to be contributed by River Mynoch (a tributary) which drains the land north of River Ystwyth watershed.

The metal content of the River Dovey system varies a great deal along the mainstream, owing to the different quantities carried by each tributary. The largest total amount of metals is found in the River Twymyn, where the zinc, copper, lead, cadmium, manganese and nickel levels are high. The lead, zinc, and copper levels are among the highest encountered in the whole region (3.7–17.6, 153–438, 1.3–3.7 µg/l respectively). The Rivers Dulas North and Dulas South are both clean with respect to zinc, copper and lead but both have a high manganese

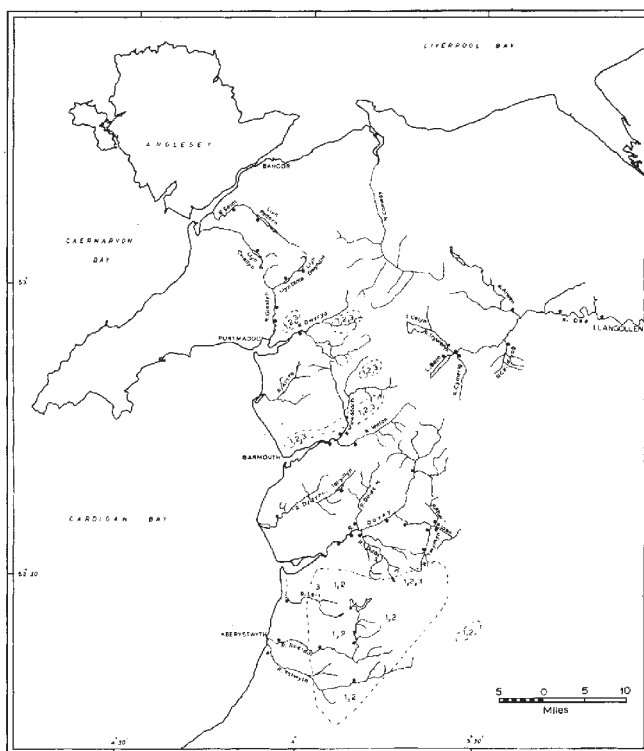


Fig. 1 Outline of the rivers and lakes studied, showing sampling points (●) and the mineralized zones (dashed line areas). 1. Lead; 2. zinc; 3. copper. (From ref 11.)

content. Some run-off into the River Dulas South from the River Twymyn watershed region, however, appears to be rich in copper, zinc, and lead.

The River Mawddach shows a high copper content (1.9–3.8 $\mu\text{g/l.}$) whereas its zinc content (14–50 $\mu\text{g/l.}$) is only just above the value for average clean water. Both copper and zinc decrease downstream owing to dilution with water from River Wnion, which is relatively cleaner. The manganese content of River Mawddach, however, is high (3–25 $\mu\text{g/l.}$). The unusually high average manganese concentrations found at the head of the Mawddach, Dwyrdd and Dysynni estuaries are possibly caused by the occasional interaction between saline water and the suspended hydrated manganese oxide carried down by the stream.

The Rivers Dwyryd and Glaslyn, which drain into Traeth Estuary and which are probably responsible for the reported high copper, zinc and manganese contents of Tremadoc Bay⁶, show slightly differing metal compositions. The River Dwyryd sampled downstream just above the tidal influence shows moderately high zinc, copper, and lead content (55.0, 3.8, and 1.4 µg/l., respectively) and unusually high cadmium and nickel content (2.6 and 3.5 µg/l., respectively). River Glaslyn shows higher zinc and manganese content than River Dwyryd but similar or lower levels of copper, lead, cadmium and nickel.

The River Dee system shows no unusually high concentrations of heavy metals except for copper, ranging between 2 and 5.5 $\mu\text{g/l.}$ Water from Lake Bala, River Tryweryn and River Alwen has a higher copper content than the streams draining the southern side of the Dee Valley. The outflows from Lake Bala, River Tryweryn and the streams draining the southern side of the Dee Valley are characterized by high manganese content. The manganese in the mainstream at Llangollen is very low due to the loss of the metal by precipitation.

The remaining streams and lakes show no unusual metal concentrations, the majority having only slightly higher levels than the clean water composition. A preliminary examination shows that high metal concentrations in the water are found in

areas of known mining history and where metal deposits are known to occur.

We thank the NERC for support.

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Received March 22; revised June 28, 1972.

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BIOLOGICAL SCIENCES

The Meaning of "Reversed Electron Flow" and "High Energy Electron" in Biochemistry

In recent years there have been some needless controversies¹ about the use of the term "high energy bond" in biochemistry. We imagine that most biochemists are perfectly aware that, when they speak of the "high energy bonds" in ATP, they do not imply that there is something exceptional about the P-O-P linkages; they mean that ATP is a compound that reacts (enzymically) with other compounds in biochemical systems, the net result being the formation of products with a lower free energy content. Some of the free energy released may be utilized in various ways but a certain fraction (usually about 25%) is dissipated as heat, and thus the reaction is virtually irreversible, whether it is anabolic or catabolic. Such irreversibility conferred by heat loss is the basis of the steady state we call "life". No one supposes that the P-O-P (anhydride) bonds enshrine the chemical potential in ATP, but Lipmann's term "high energy bond" remains a useful metaphor for at least two reasons. First, the phosphate group can be readily attached to all kinds of biochemical compounds and is therefore an excellent intermediary in linking one reaction with another. Secondly, the free energy of hydrolysis of the P-O-P anhydride bond at pH 7 is relatively high. Thus a reaction such as



with an equilibrium lying to the left can be reversed by coupling it enzymically with ATP hydrolysis:



in which the equilibrium is to the right.

While the term "high energy bond", properly understood, need cause no ambiguity, the use of the term "high energy electron" (see ref. 2) to describe biochemical redox potentials lower than -0.32 V at pH 7 seems to involve much greater dangers. First, high energy electrons—those moving at relativistic velocities—do exist, but they are certainly not the electrons described. Second, the known biochemical spectrum of redox potentials at pH 7 stretches, without any marked break, all the way from $+0.8$ V to about -0.6 V; to impose a "break" at -0.32 V merely because this happens to be the

redox potential of the most common anaerobic electron carriers, seems to be not only entirely arbitrary but also invites misinterpretation by physical chemists.

Recent conversations with chemists working on the fringe of metabolic biochemistry have alerted us to the dangers of another term now becoming familiar in the biochemical vocabulary: "reversed electron flow". The concept of reversed electron flow stems from a series of papers originating from Professor Chance's laboratory in 1961³⁻⁵. Under normal aerobic conditions in mitochondria, the reaction



proceeds from left to right. This is not because the equilibrium of the reaction lies in that direction, but because the enzyme-bound FADH_2 is rapidly re-oxidized by the cytochrome chain and the concentration of one of the products is therefore always low. In fact the pK of the reaction ≈ 0 , so that if an excess of fumarate is added to an isolated system the reaction will proceed to the left. This is, in a sense, "reversed electron flow" brought about by increasing the concentration of one of the products of a biochemical reaction which, *in vivo*, normally runs in the opposite direction. (An increased concentration of FADH_2 would be equally effective, an increase of both fumarate and FADH_2 would be more effective still.) We should like to assure our chemical colleagues that this is not what biochemists mean by "reversed electron flow".

Consider the reaction



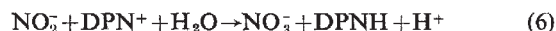
Because the E_0' of the DPN^+/DPNH couple is -0.32 V, the equilibrium lies far to the left. If we make an approximation that a ten-fold increase in concentration is equivalent to 30 mV change in electrode potential which itself involves a free energy change of 1.4 kcal per electron pair, it is clear that the reaction proceeds almost entirely to the left with a pK of about 11 and a free energy loss of about 15 kcal. No appreciable change in the normal direction of electron flow can possibly be brought about by altering the concentrations of the reacting species because it would be necessary to add succinate and DPN^+ in concentrations each $10^{5.6}$ greater than those of fumarate and DPNH .

The reaction can be reversed, however, by coupling it to ATP hydrolysis. This coupled reaction was in fact one of the reactions that Chance and Hollunger studied:



If sufficient ATP is present, the reaction should be reversible; this would be achieved when $n=2$, that is 16 kcal energy are available from ATP hydrolysis. The experimentally determined value of n proved to be approximately 2.5⁶. This is an example of "reversed electron flow", though naturally we do not know to what extent it takes place *in vivo*.

A reaction that must take place *in vivo* because it is essential to the survival of *Nitrobacter* is



The reaction, which again involves reversed electron flow, does take place⁹ but only when "driven" by ATP hydrolysis. *Nitrobacter* is commonly grown in 10^{-3} M NaNO_2 . If the DPN^+/DPNH ratio were to be maintained in the region of unity by mere concentration differences of nitrite and nitrate, then the nitrite concentration required to achieve this ratio would be 10^{12} M. (Solid sodium nitrate is about 32 M.)

We hope we have made it clear that, though the phrase "reversed electron flow" is often applied to reactions such as (5) or (6), it is a figure of speech, implying processes quite different from the concentration-dependent reversal of electron flow seen in reaction (3). We have seen the hopeless misunderstanding that has arisen over the simple term "high energy bond" and we fear the same misunderstanding in connexion

with "high energy electron". We wish, therefore, to prevent a similarly sterile debate over "reversed electron flow".

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Use of a New Detector for X-ray Diffraction and Kinetics of the Ordering of the Lipids in *E. coli* Membranes and Model Systems

ALTHOUGH X-ray diffraction is the most powerful tool for the direct determination of structural parameters, long exposure times limit its use. Using a new detector we have been able to shorten the exposure times by several orders of magnitude, thus allowing study of unstable samples and of the kinetics of structural transitions. We have applied this technique to biological membranes and model systems.

The new counter (to be described in detail elsewhere) is a linear, position sensitive proportional counter developed from designs by Borkowski and Coop¹. The anode is a high resistance wire parallel to the entrance window, and the position of an electron pulse can be located with an accuracy of 0.2 mm over a length of 20 cm, by using the difference in integration of the pulse as it reaches the two ends of the wire.

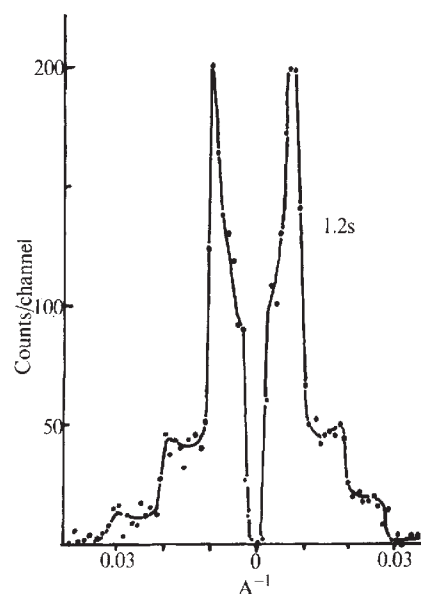
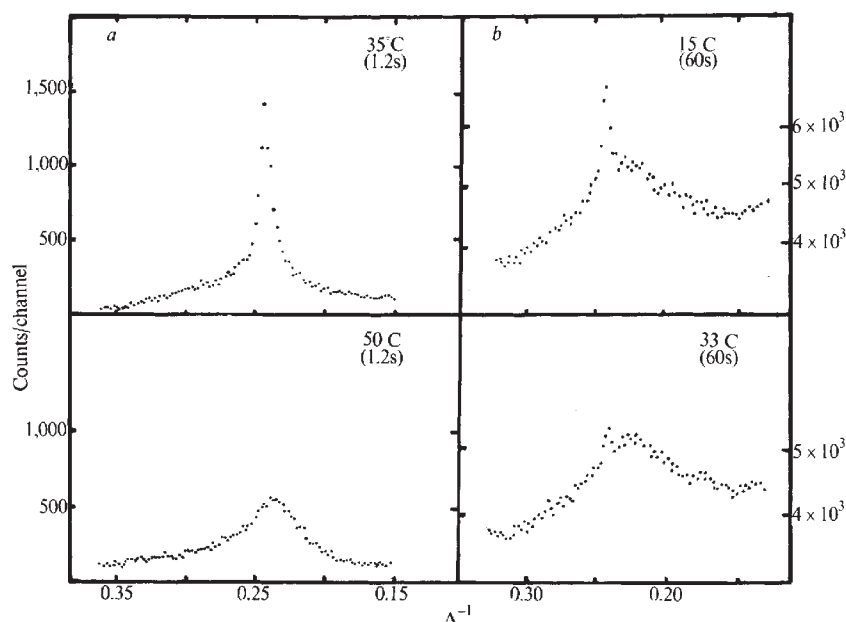


Fig. 1 Small angle X-ray diffraction of a phosphatidic acid-lysozyme-water phase, displaying a long range organization of the lamellar type with repeat distances of $97 \text{ \AA}^8$. The counter is 6 cm long, with a vertical aperture of 8 mm and a thickness of 6 mm, filled with xenon at one atmosphere. The quantum efficiency is 60%. The X-ray source is a copper tube operated at 30 kV, 20 mA. The beam is focused by a bent gold plated glass mirror. The distance between the sample and the counter is 25 cm. The spectrum was obtained in 1.2 s.

A pulse shape analysis circuit converts the shape difference to a time difference, which is subsequently converted to a voltage amplitude scale. The output is connected to a multi-channel analyser, and, on the memory display, the intensity spectrum of the scattered X-rays can be followed during collection. Counting rates of up to 2.0×10^4 c.p.s. can be obtained without loss of spatial resolution. Counting losses are uniform over the whole spectrum, thus changing only the normalization. In our case the losses were caused by the multichannel analyser.

Spectra obtained with lipid-water systems, lipid-protein-water systems and *E. coli* membrane vesicles are shown in Figs. 1 and 2. The times needed to obtain these spectra are about two orders of magnitude less than those involved if

Fig. 2 High angle X-ray diffraction of dipalmitoyl lecithin-water lamellar phase (a) and *E. coli* membrane vesicles (b) at temperatures above and below the order-disorder transition of the paraffin chains. The membrane vesicles were prepared according to Kaback⁹. The X-ray source and the counter are the same as in Fig. 1. The X-ray beam is focused with a bent quartz monochromator. The distance between the sample and the counter is 10 cm.



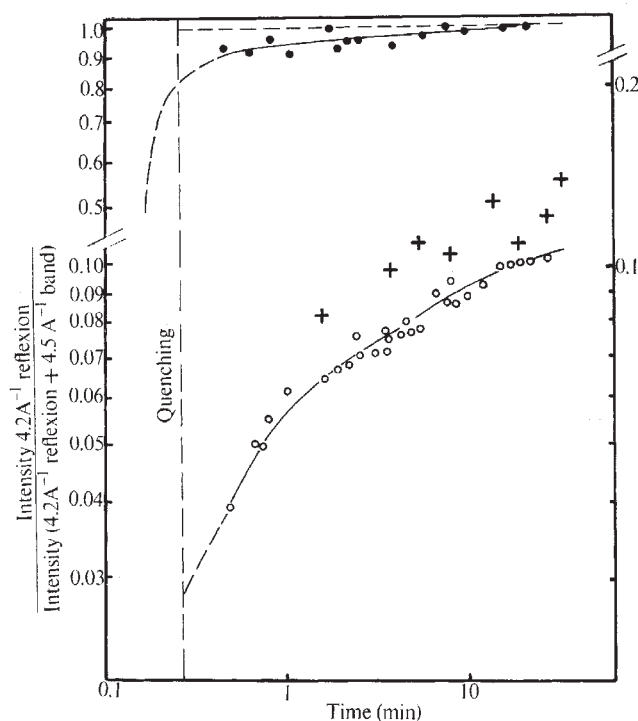


Fig. 3 Kinetics of the disorder→order transition of the paraffin chains of the lipids in: ○, lamellar phase of a phosphatidic acid-lysozyme-water system displaying hydrophobic lipid-protein interactions⁸; +, *E. coli* membrane vesicles; ●, phosphatidic acid-water lamellar phase. The samples, 1 mm thick, mounted in a tight holder between two thin beryllium plates, are first allowed to equilibrate for 30 min, 10° C above the transition temperature, and then quenched in water for 15 s, 10° C below the transition temperature, and are placed in the X-ray beam in the sample holder set at the quenching temperature. The measurements start 30 s after the start of the quenching. For the model systems the first four measurements last 6 s and are spaced by 1 s; for the *E. coli* membrane vesicles longer exposure times have been used. After 4 measurements, 30 s were needed to record the spectra on a digital plotter.

conventional film methods are used, owing to the absence of the chemical background of the film. Two additional features should be mentioned; first, that the information is seen as it is being collected, and second, successive spectra can be routed electronically to different parts of the memory.

Previous studies carried out have revealed the existence of order-disorder transitions in the paraffin chain region in some lipid-protein-water systems (L. Mateu, to be published) and in *E. coli* membrane vesicles^{2,3}. The new counter made it possible to follow the kinetics of the transitions, and, in some cases, to reveal the existence of hysteresis. Little is known about these transitions in biological membranes. Although it seems that under physiological conditions the paraffin chains are disordered, and that a transition to the ordered state inhibits the physiological activities²⁻⁴, it has been suggested that in some instances a local transition in the organization of the paraffin chains might play a physiological role^{5,6}.

The kinetics of the disorder→order transition are shown in Fig. 3. The degree of organization of the paraffin chains is measured by an operational parameter, the ratio of the intensity of the 4.2 Å⁻¹ reflexion over the total intensity of both the 4.2 Å⁻¹ reflexion and the 4.5 Å⁻¹ band⁷. The transition is too rapid to be followed in homogeneous synthetic lipids; it is somewhat slower in heterogeneous natural lipids, and surprisingly slow in the lipid-protein-water system. The kinetics of the transition in *E. coli* membrane vesicles is similar to that of the lipid-protein-water system. The reverse transition, namely the melting of the chains induced by a rise of temperature, is completed within a few seconds and could

not be followed. In order to explain the observed kinetics, it is reasonable to assume that the disorder→order transition, contrary to the order→disorder one, involves, in systems containing heterogeneous lipids, a segregation of the lipids according to the type of the paraffin chains. The fact that the diffusion is much slower in the protein-lipid-water systems and *E. coli* membrane vesicles than in lipid-water systems containing heterogeneous lipids indicates that the proteins strongly hinder the diffusion of the lipids. We checked that this was also true above the transition temperature; when all the paraffin chains were melted, a cold lipid-protein-water sample was heated 10° C above the transition temperature for only 15 s and then quenched; the kinetics of the ordering proceeds faster under these conditions than when the sample has been allowed to equilibrate a long time at high temperature before quenching. This indicates that after 15 s in the melted state the lipids are not yet fully randomized within the phase. The only data relating to the lateral diffusion of lipids reported in the literature concern liposomes. By electron spin resonance, a value of 10⁻⁷ s has been measured for the exchange frequency between two neighbouring lipids (P. Devaux and R. M. McConnell, private communication). Such a value should give a complete randomization of the lipids within the phase in a few seconds, which is not what we observe in the lipid-protein-water system. It confirms that in this system, and in *E. coli* membrane vesicles, the diffusion of the lipids is considerably slower than in liposomes.

The other set of experiments on the same systems deals with the order-disorder transition followed in a static way as a function of temperature. The data are shown in Fig. 4. Significant hysteresis was observed for the lipid-protein-water system and for *E. coli* membrane vesicles, whereas the hysteresis was much smaller in the natural heterogeneous lipid-water system. The complete hysteresis cycle was described at least twice in each sample.

The properties of the new counter illustrated here should allow numerous applications in the biological field, especially in the case of naturally ordered material under different physiological conditions, that is, retina and muscle.

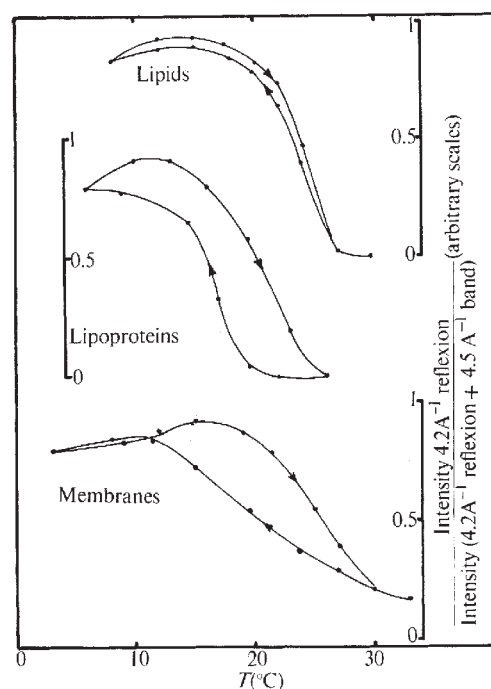


Fig. 4 Hysteresis of the order-disorder transition of the paraffin chains as a function of temperature, for the same samples as in Fig. 3. The time between two successive measurements was 7 min, and the temperature steps 4° C. Longer delays were added at the highest and the lowest temperatures.

We thank Dr V. Luzzati for discussion and Dr L. Mateu for communication of his unpublished data.

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Effects of *Polymorphus* (Acanthocephala) on Colour and Behaviour of *Gammarus lacustris*

IN 1969–70 1,420 adult specimens of the amphipod *Gammarus lacustris* were taken from 2 eutrophic ponds in the northern Zealand; 94.9% were normal in colour (grey to brown) and 5.1% were blue. Dissections showed that all the blue shrimps were infected with the final larval stage (cystacanth) of the acanthocephalan genus *Polymorphus*, whereas the brown shrimps were only 3.9% infected with cystacanths. This difference was statistically highly significant ($\chi^2 = 860.2$, $P < 0.001$).

Two of these parasites were successfully reared to adults in a domestic duck and were identified as *Polymorphus minutus*. Measurements of the proboscis hooks of 12 larvae indicated that these were probably *P. minutus*.

Occurrence of the blue shrimps varied throughout the year; the first few specimens occurred in May and the maximum number was reached in June–July simultaneously with the highest infection. No blue shrimps were found during the winter.

On air drying at room temperature the brown shrimps turned red; the blue specimens turned pale, suggesting a lack of carotenoid pigment. To test this possibility the total carotenoid contents of 13 blue and 28 brown male shrimps were extracted. Because of indications of a fluctuation in the carotenoid content of the ovaries correlated with the reproduction cycle, female shrimps were not studied. Before extraction the parasites were removed from infected shrimps. Spectrophotometric examination of the extracts showed a smaller amount of carotenoids/mg wet weight in the blue shrimps as compared with the brown shrimps ($P = 0.016$ by the binomial test).

Using a dissection needle I punctured the shrimps dorsally under water and the colour of the outflowing haemolymph was estimated by eye. From 625 uninfected brown shrimps the colour distribution was 59.8% blue, 13.1% bluish-green and 27.0% green. From 20 brown shrimps, harbouring cystacanths, the frequency of the blue haemolymph was higher (90%) ($\chi^2 = 7.4$, $0.01 > P > 0.005$). The frequency of the blue haemolymph among 9 brown shrimps infected with the growth stage (acanthella) of the parasite, on the contrary, did not differ significantly from the uninfected shrimps ($\chi^2 = 0.015$, 0.70

$> P > 0.50$). All blue shrimps contained blue haemolymph. Their colour was lost when all the haemolymph was removed, indicating that the abnormal colour was due to blue haemolymph seen through an unpigmented cuticle. The brown specimens, on the contrary, did not change colour on removal of the haemolymph, owing to the normal pigmentation of the cuticle masking the colour of the haemolymph.

The red colour of the cystacanth of *P. minutus* has previously been shown to be due to esterified astaxanthin derived from the shrimps¹. My results show that the larvae of *P. minutus* affect the amount and distribution of the carotenoids of *G. lacustris*.

A colour change of the shore crab *Carcinus maenas*, due to carotenoid intake by the rhizocephalian parasite *Sacculina carcini*, has previously been described².

Because of the contrast of their light blue colour to the dark bottom of their ponds, the blue shrimps were easily seen when swimming in the surface water. The preferred occupation site of the shrimps was tested in the laboratory. A total of 18 infected blue and 26 uninfected brown shrimps were placed in an aquarium with a light exposed top and a dark bottom. The bottom was provided with bottom material and *Fontinalis* sp. from the ponds; this resembled the conditions in the natural habitat. The shrimps inhabiting the light exposed area were counted 50 times with intervals of 30 seconds, and after every 10 countings the shrimps were disturbed by stirring. The dark bottom was most frequently inhabited by both the blue and the brown shrimps but in the light exposed top area the blue shrimps were observed 81 times more frequently than the brown ones.

By varying the light and dark areas I have confirmed that blue shrimps show a higher positive phototropism.

Denny³ found that *G. lacustris* infected with *Polymorphus paradoxus* were found more frequently among shrimps clinging to floating objects than among shrimps caught from the bottom. In this respect *P. paradoxus* resembles *P. minutus* but Denny does not mention any colour changes.

The behaviour of the blue *G. lacustris* increases the chance of the parasites' reaching their final hosts (waterbirds). This was shown by placing a domestic duckling in an aquarium to which were added 11 blue (infected) and 39 brown uninfected shrimps selected from a July sample, giving a distribution of infected shrimps similar (22%) to that found in the sample. After about half of the blue shrimps had been eaten the duckling was removed and the residual shrimps were counted. The result showed that 7 blue and 10 brown shrimps were eaten, that is, the chance of being eaten was 2.5 times greater for the blue than for the brown shrimps ($\chi^2 = 5.5$, $0.025 > P > 0.020$).

I thank Dr D. W. T. Crompton for his help.

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Received March 9; revised April 17, 1972.

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Effects of Alkali Metal Chlorides on Activity in Rats

LITHIUM salts are widely used in the clinical control of manic states¹ and also have effects on animal behaviour, suppressing vertical rearing activity in rats² and reducing aggression in several species^{3–5}. In contrast, salts of rubidium increase activity and aggression in mice and monkeys^{6,7}, leading to the suggestion that rubidium might be useful in the clinical

Table 1 Effects of Alkali Metal Chlorides on Vertical Rearing Activity (Arbitrary Units) in Male Rats

	LiCl	NaCl	KCl	RbCl	CsCl
Mean score	7.98	37.25	43.43	71.58	53.18
s.d.	10.41	24.68	26.02	36.58	27.81
Percentage deviation from NaCl score	-78.58	—	+16.59	+92.16	+42.77
Difference from NaCl score: $P <$	0.01	—	n.s.	0.01	0.05

treatment of depression⁸. In the present experiment I compared the effects of five alkali metal chlorides on two types of activity in rats—vertical rearing and horizontal locomotion.

I studied ten 100-day-old male rats, F_1 hybrids of the Roman High and Low Avoidance strains, kept in a vertical transparent tube 46 cm high with an internal diameter of 23 cm. The ceiling and floor of the tube formed the two plates of a capacitor which was linked to a proximity meter⁹ whose output was linearly related to the distance between the ceiling and the head of a rat placed in the tube. The animal's rearing activity was thus registered on a pen recorder, total activity in a vertical direction being proportional to the area beneath the curve (measured by feeding the output of the proximity meter into a d.c. integrator). Horizontal movements of the animal caused the floor to tip about a central pivot, triggering four micro-switches at the edge of the floor. Total switchings gave an index of horizontal locomotory activity. Each animal was tested in the apparatus for 5 min.

Lithium, sodium, potassium, rubidium and caesium chlorides were examined, each being administered in doses of either 1 or 3 meq/kg, dissolved in "Water for Injection" BP and injected intraperitoneally (0.5 ml./100 g body weight). Injections were given 15 min before a 5 min test period. Subjects were tested daily over 10 days, receiving on each day a different drug and dosage combination. After a 7 day interval the experiment was repeated, using the same subjects, over a further 10 day period. Each subject thus experienced each treatment condition twice, the order of treatments being independently randomized for individual animals. The experimental design was therefore a 5 (drugs) $\times$ 2 (doses) $\times$ 2 (replications) factorial with repeated measures on all three factors.

Analysis of the vertical activity data revealed a statistically significant drugs effect ($P < 0.001$), neither the use of different drug dosages nor the replication of the experiment causing significant changes in vertical activity. Using a pooled error term in the analysis of variance and applying Dunnett's test¹⁰ for all comparisons with a control, it was shown that, as compared with the sodium chloride-treated animals, rats given lithium chloride exhibited less rearing ($P < 0.01$), while rubidium chloride ($P < 0.01$) and caesium chloride gave elevated rearing scores ($P < 0.05$). Potassium chloride apparently increased vertical activity but the effect was not statistically significant. No interaction term in the analysis of variance attained significance at the 5% level. The vertical activity data, averaged over subjects, doses and replications, are represented as percentage deviations from the scores of sodium chloride-treated subjects (Table 1).

The only significant finding for horizontal locomotory activity was an increase in the activity of rubidium chloride-treated rats above the scores for animals given sodium chloride ($P < 0.01$). An increase in the horizontal activity of subjects given caesium chloride just failed to attain significance at the 5% level. Neither lithium nor potassium chlorides caused deviations in horizontal activity from sodium chloride levels, nor was there any effect due to the use of different dosages or to the replication of the experiment.

These findings confirm those previously reported for lithium chloride^{2,11}. Rearing activity, in these experimental conditions, has been shown previously to differ from horizontal activity

in being strongly influenced by environmental stimuli, and the reduction in vertical activity by lithium chloride has been related to a selective suppression of behaviour under stimulus control, resulting from a lithium-induced disruption of central sensory analysis¹¹.

This work shows that rubidium and caesium chlorides may be regarded as enhancing or facilitating stimulus analysis, but as they also elevate horizontal activity scores it is equally possible that other mechanisms are operating. A generalized state of heightened arousal may be produced as a result of drug-induced noradrenaline release in the brain⁸. A change in muscular competence may occur following the activation by rubidium and (to a lesser extent) by caesium of electrogenic sodium pumping¹².

This preliminary study clearly suggests that alkali metal ions may markedly interfere with the physiological mechanisms underlying behaviour. Further work is needed to elucidate the nature and extent of this involvement and to determine the value of the use of alkali metal salts in the clinical treatment of the affective disorders.

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Received January 25, 1972.

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Colour Selectivity in Motion After-effect

STATIONARY lines appear to move upwards after prolonged visual exposure to downward moving lines. The experiment reported here shows that the movement after-effect is appreciably diminished when the moving lines observed during inspection are seen in red light and the stationary test lines are viewed in green light. We expected to obtain colour selectivity in the movement after-effect following the demonstration of motion-specific colour after-effects by Hepler¹. She found that alternate exposure to downward moving green lines and upward moving red lines caused downward moving lines later seen in white light to appear red and upward moving lines green. Hepler proposed that motion detectors in the human visual system are also selective to wavelength so that during exposure to one stimulus only the neural analysers tuned to both the specific direction of motion and the wavelength are excited. Because these analysers adapt during inspection and are suppressed for a period of time afterwards, the downward moving lines presented in white light as the test stimulus are signalled via downward-selective motion detectors tuned to the opposite colour. McCollough² has offered a similar explanation of colour after-effects which are specific to contour orientation.

In our experiment, motion after-effect was measured with the inspection (moving) and test (stationary) contours both red (Wratten filter 33) or both green (Wratten filter 61), or with one red and the other green. The inspection pattern sub-

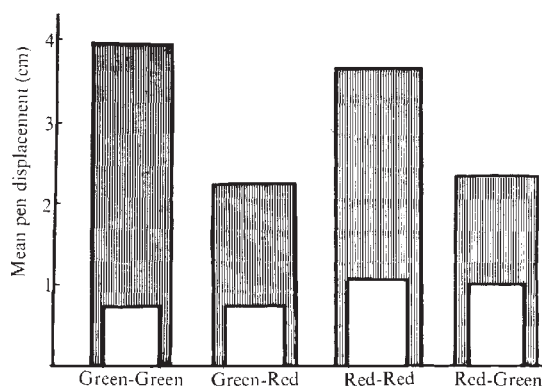


Fig. 1 Effect of stimulus colour on the motion after-effect in monoptic (shaded columns) and dichoptic (white columns) viewing conditions.

tended $1^{\circ} 44'$ in diameter, and was a square-wave grating of $1.1 \text{ cycles degree}^{-1}$ displayed on an oscilloscope face. The grating moved from left to right at $2.5 \text{ cycles s}^{-1}$, and was shown for 4 min on the first inspection trial in a given condition and for 1 min on subsequent trials. The test grating was the same size and spatial frequency. Because the experiment was conducted in a darkened room it was necessary to have a patterned background (stripes illuminated by diffuse white light) to the test grating to induce a motion after-effect³. The background was constant across the test conditions. A tracking method³ was used to measure motion after-effect. After the inspection period, the subject moved a pen on a carriageway laterally at the speed at which the test grating appeared to be moving. The pen left a trace on paper moving at 0.25 cm s^{-1} , at right angles to the direction of the carriage, and the amount of after-effect at different periods after inspection was given by the slope of the trace.

We compared motion after-effect in conditions of monoptic and dichoptic viewing. Inspection of moving contours in white light with one eye results in a reduced after-effect when the test pattern is seen with the other eye⁴. The motion-specific colour after-effect reported by Hepler¹ does not, however, show interocular transfer⁵. We therefore expected that colour selectivity in motion after-effect would occur with monoptic but not dichoptic display of contours. Eight subjects were each tested over four sessions. In a single session viewing was either monoptic (right eye alone used) or dichoptic (inspection lines seen by left eye and test lines by right eye), the inspection colour was either red or green, and eight test judgments (four with the stationary lines seen in red light, and four in green light) were made. The testing sequence was counterbalanced across subjects.

The mean lateral movement (in mm) of the pen in a 15 s test period is shown in Fig. 1 for the two viewing conditions and the four combinations of inspection and test colours. An analysis of variance indicated that dichoptic after-effects were reduced relative to monoptic, $F(1, 7) = 12.68$, $P < 0.01$. Neither the inspection colour, $F(1, 7) = 0.05$, $P > 0.05$, nor the test colour, $F(1, 7) = 0.87$, $P > 0.05$, was significant, but the interaction between the inspection and test colours, $F(1, 7) = 22.85$, $P < 0.01$, and between viewing condition, inspection colour, and test colour, $F(1, 7) = 14.24$, $P < 0.01$, were significant. Multiple comparisons revealed that the motion after-effect was larger in monoptic conditions when the inspection and test gratings were identical in colour than when they were different. Colour selectivity was not found, however, with the motion after-effect under dichoptic conditions.

The results of this experiment can be incorporated into a model⁶ attributing the movement after-effect to post-excitatory suppression of neural feature detectors responsive to the rate and direction of image motion. Support is given to the claim by Hepler¹ that the human visual system contains motion analysers which are also selective to wavelength. The finding

that interocular transfer of spatial information occurs (although diminished) but colour selectivity is no longer maintained suggests that motion analysers tuned to wavelength are exclusively monocular, whereas some (if not all) motion detectors which lack colour specificity are binocular.

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Requirement for θ -Bearing Cells in Lymphocytic Choriomeningitis Virus-induced Central Nervous System Disease

THE immunopathological basis of the acutely fatal convulsive central nervous system (CNS) disease produced in adult mice following cerebral infection with lymphocytic choriomeningitis (LCM) virus is well established¹. Thus, a variety of immunosuppressive treatments, given to adult mice inoculated intracerebrally with a potentially lethal dose of virus, produce a transient or permanent ablation of both the histological and clinical correlates of LCM. Permanently protected mice usually maintain high virus concentrations in their brains and blood for life^{2,3}, presenting a picture which, on virological grounds, is strikingly similar to the lifelong virus carrier state arising in mice inoculated with LCM virus shortly after birth^{3,4}.

We have already shown that a single dose (150 mg/kg) of cyclophosphamide ('Cytoxan', Mead Johnson) given to 10–12 week old BALB/c mice 3 days after an intracerebral (i.c.) inoculation with 1,000 LD₅₀ of the Armstrong strain (E-350) of LCM virus converts about 85% of these animals to permanent virus carriers^{4,6}. Furthermore, when cyclophosphamide (CY)-induced carrier mice are grafted with syngeneic spleen cells from donors rendered immune to LCM virus, CNS pathology and disease typical of LCM are regularly produced. These occur in two main forms depending on the duration of the CY-induced virus carrier state before adoptive immunization. Mice with infections of relatively short duration (up to about 2 weeks) all develop an acutely fatal CNS disease within 7–10 days, preceded by characteristic histological evidence of choriomeningitis. No lesions are seen in other CNS areas, and animals develop minimal or no evidence of circulating complement-fixing (CF) antibodies. By contrast, most mice with longer term CY-induced carrier infections survive adoptive immunization although transient CNS symptoms of two to three days duration usually occur. Accompanying these symptoms, which begin about seven days after cell transfer, animals show, histologically, an attenuated form of choriomeningitis and, in addition, approximately 50% display a necrotic lesion of the cerebellar granule cell layer. Such animals also develop very high levels of circulating CF antibodies during the second week after cell transfer. Minimal or no evidence of LCM is produced in CY-induced carrier mice given virus-specific antibody or spleen cells from non-immunized donors.

The response to adoptive immunization of mice whose carrier states are established by neonatal inoculation of virus presents yet another contrast. These animals fail to develop either overt CNS disease or any detectable histological evidence of CNS pathology following adoptive immunization, but they develop CF antibodies in high titre^{5,6}

These differing responses to adoptive immunization raise some question as to what role, if any, antibody plays in mediating disease and CNS pathology.

In an attempt to answer this question we have used heterologous anti-BALB/c brain associated θ serum⁷ to determine whether thymus-dependent (T) lymphocytes in populations of immune donor spleen cells are necessary: first to elicit the histological and/or clinical correlates of LCM in CY-induced carrier recipients, and second to enable mice with longer term carrier states, established by CY or neonatal infection, to produce virus-specific CF antibodies.

Anti- θ serum was produced in rabbits by the method of Golub⁷, heated (56° C for 30 min), and four volumes absorbed 4 times with one volume of packed BALB/c erythrocytes. Trypan blue exclusion⁸ was used as a measure of the relative cytotoxicity of this antiserum for thymic and splenic lymphocytes. In the presence of guinea-pig complement (C'), 0.5 ml. of 1/5 dilution of anti- θ serum in Eagle's medium (MEM), incubated at 37° C for 30 min with an equal volume of MEM containing 10×10^6 thymocytes or 100×10^6 spleen cells, killed

98–100% and 30–35% respectively. Substitution of identically treated normal rabbit serum (NRS) for anti- θ serum resulted in 5–10% cytotoxicity for both splenic and thymic lymphocytes.

Spleen cell suspensions from adult BALB/c mice of either sex were obtained 5–10 days after the animals received the last of four or five weekly intraperitoneal (i.p.) injections of LCM virus (approximately 10^4 i.c. LD₅₀) and were treated with either normal or anti- θ serum. After incubation, cells were washed and pooled in MEM and inoculated i.p. into the different types of carrier mice.

The occurrence of illness and death in CY-induced carriers after cell transfer is shown in Table 1. All mice with infection of 9 days duration developed fatal disease after receiving 100×10^6 spleen cells from LCM virus-immune donors pre-incubated in NRS and C' or MEM. A ten-fold reduction in the number of untreated spleen cells (10×10^6) transferred did not impair their ability to mediate transient CNS disease although mortality was reduced to about 50%. By comparison, treatment of 100×10^6 spleen cells with anti- θ serum and C' rendered them incapable of producing CNS disease, even though at least 65% of cells were viable at the time of transfer. Animals with a CY-induced carrier state of 21 days duration failed to develop the usual transient CNS symptomatology after receiving 100×10^6 spleen cells pretreated with anti- θ serum and C'.

Table 2 shows the effect of T-lymphocyte depletion on the

Table 1 Effect of Anti-BALB/c Brain-associated θ Serum on the Ability of LCM Virus-immune Donor Lymphocytes to Mediate CNS Disease in LCM Virus Carrier Mice*

Immune spleen cells		CY-induced carrier state				Neonatally-induced carrier state	
Dose	Treatment †	Disease (9 days)	Death (9 days)	Disease (21 days)	Death (21 days)	Disease	Death
None	None	0/8	0/8	0/10	0/10	0/10	0/10
100×10^6	None	9/9	9/9	4/5	1/5	0/5	0/5
100×10^6	NRS + C'	7/7	7/7	8/10	1/10	0/10	0/10
100×10^6	Anti- θ + C'	0/7	0/7	0/10	0/10	0/10	0/10
10×10^6	None	6/8	3/8	nd ‡	nd	nd	nd

* CY-induced carriers: 12 week-old male BALB/c inoculated i.c. with 1,000 LD₅₀ of LCM virus and given CY (150 mg/kg) 3 days later. Neonatally-induced carriers were inoculated, i.c. with 1,000 LD₅₀ of LCM virus within 24 h of birth. Immune spleen cells were obtained 5–10 days after adult male BALB/c mice received the last of 4 or 5 weekly i.p. injections of 10^4 i.c. LD₅₀ of LCM virus.

† Aliquots of spleen cells suspended in 0.5 ml. of Eagle's medium (MEM) were pre-incubated at room temperature for 1 h with an equal volume of normal rabbit serum (NRS) or anti- θ serum (1 : 5 dilution). Cells were centrifuged, suspended in 1.0 ml. of a 1 : 7 dilution of guinea-pig complement (C'), incubated for 30 min at 37° C, re-sedimented, pooled in MEM, and inoculated i.p. into carrier recipients. Untreated cells were inoculated without pre-incubation or C' addition.

‡ Not done.

Table 2 Effect of Anti-BALB/c Brain-associated θ Serum on the Function of LCM Virus-immune Donor Lymphocytes Transferred to LCM Virus Carrier Mice*

		CNS pathology		Viraemia †	CF antibody mean/range ‡	CNS viral antigen †	CNS-bound IgG †
Treatment		LCM	Cerebellar necrosis				
CY-induced carrier state (21 days)							
Control (no cells)	None	0/10	0/10	2.7	0/0		
100 × 10 ⁶ immune cells	NRS + C'	8/9	4/8	0.5	1,500/200–12,000	Decreased	Increased
100 × 10 ⁶ immune cells	Anti-θ + C'	0/10	0/10	0.7	100/25–200	Decreased	Increased
Neonatally-induced carrier state (132 days)							
Control (no cells)	None	0/8	0/8	4.0	0/0		
100 × 10 ⁶ immune cells	NRS + C'	0/10	0/10	0.5	3,000/200–12,000	Decreased	Increased
100 × 10 ⁶ immune cells	Anti-θ + C'	0/10	0/10	3.3	0/0–25	Decreased	Increased

* Production of virus carrier mice and immune lymphocytes is described in Table 1. CY-induced carriers and neonatally induced carriers were killed, respectively, 12 and 17 days after adoptive immunization.

† Observations based on 3–6 mice. Viraemia: mean LD₅₀ determined by intracerebral titration of individual plasma samples in adult mice. CNS viral antigen: relative intensity of immunofluorescent viral antigen in sections of brain using an LCM virus-specific antiserum (rabbit) conjugated with fluorescein isothiocyanate. CNS-bound IgG: relative intensity and location of fluorescence after straining of washed brain sections with a commercially prepared ('Melpar') rabbit anti-mouse IgG conjugate. Brain sections from normal mice showed no fluorescence.

‡ Mean titres of groups of 8–10 mice.

ability of transferred cells to mediate CNS pathology and to elicit CF antibody in mice with CY-induced carrier states of 21 days or in carriers produced by neonatal infection. The production of antibody is correlated with a marked decrease in viraemia and a moderate decrease in immunofluorescent-stainable viral antigen in the brain, together with a relative increase in the amount of immunofluorescent-staining CNS-bound IgG. CY-induced carrier mice adoptively immunized with 100×10^6 immune spleen cells pretreated with anti- θ serum failed to develop histological evidence of CNS disease when examined 12 days after cell transfer. Such carriers, given an equivalent spleen cell dose pretreated with NRS and C', showed typical choriomeningitis and about 50% had developed focal areas of necrosis in the cerebellum. As expected on the basis of previous studies^{5,6}, carrier mice produced by neonatal infection developed neither CNS disease nor CNS pathology after adoptive immunization.

Although treatment of spleen cells with anti- θ serum rendered them incapable of eliciting LCM, it did not eliminate their ability to produce an antibody response. Significant levels of circulating CF antibody were still detectable in the long-term CY carrier and bound IgG was readily demonstrable in the membranes and parenchyma of the brains of both carrier types which had received T-lymphocyte depleted spleen cells. As immunofluorescent staining revealed both viral antigen and IgG to be located at identical sites in the CNS, it is possible that the observed decrease in viral antigen may have been the result of "blocking" due to complexing with antibody. This possibility is supported by our inability to show a significant decrease in infectious virus in brains of adoptively immunized carriers, although such a decrease has been reported to occur⁹ in somewhat different experimental conditions. The failure of spleen cells treated with anti- θ serum to produce circulating CF antibody levels in the neonatally inoculated carrier comparable with those in the CY-induced carrier may be related to the initially greater levels of circulating virus in neonatal carriers which might mask the presence of antibody. This possibility has been discussed elsewhere⁶.

Although these results do not define the relative role of T-lymphocytes in the expression of both cell-mediated and humoral immune responses to LCM virus, they clearly indicate the requirement for θ -bearing lymphocytes for the production of classical LCM. Furthermore, the development of circulating CF antibody, the apparent presence of virus-antibody complexes in the brain, and absence of pathology in CY-induced carrier mice which received spleen cells depleted of T-lymphocytes strongly argue against the role of immune complexes in mediating CNS disease.

We thank Mr Gary Cohen and Mr John Hodous for expert technical assistance. This work was supported in part by research grants from the National Institute of Allergy and Infectious Diseases and career development awards from the National Institute of Neurological Diseases and Stroke, US Public Health Service. Dr Prendergast is a Professor of Research to Prevent Blindness, Inc.

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Senescence of an Antibody-forming Cell Clone

SELECTION and propagation of a single antibody-forming cell clone has been effected by serial transfer of limited numbers of spleen cells into irradiated syngeneic mice¹. Original spleen cell donors were immunized with DNP conjugated to bovine gamma globulin (BGG). A single clone was identified by its characteristic secreted antibody to DNP. Sera of recipient mice were analysed by isoelectric focusing on polyacrylamide gels, and specific antibody molecules were visualized by ¹²⁵I-hapten binding and autoradiography². Using the antibody isoelectric spectrum as a phenotypic marker, we were able to follow one clone of anti-DNP forming cells (E9) through successive transfer generations; at the time of our first report¹, clone E9 had been propagated through five transplant generations.

Continuing serial transfer showed that clone E9 had a finite life span. The evidence presented here suggests that we are dealing with clonal senescence *in vivo*, and that the progenitor cells of clone E9 had a limited potential for proliferation. Recipients of spleen cells from an E9 donor at each generation, up to and including the seventh transfer, consistently exhibited the characteristic isoelectric spectrum of E9 anti-DNP molecules in their serum, without other antibodies to DNP³. The identity of the E9 clonal product at the third and seventh transfer generations is illustrated in Fig. 1a and b. Sera of recipients of the eighth transfer contained either trace amounts of E9 antibody only, or lacked anti-DNP molecules. After injection of further antigen, the hosts developed a heterogeneous anti-DNP pattern in which the presence of E9 antibody could not be detected.

The loss of E9 production was not an abrupt event at the eighth transfer. The relative production of E9 antibody at each generation was measured by titration of anti-DNP in the sera using a modified Farr procedure⁴. Fig. 2 shows the maximum E9 antibody production achieved in any recipient at each

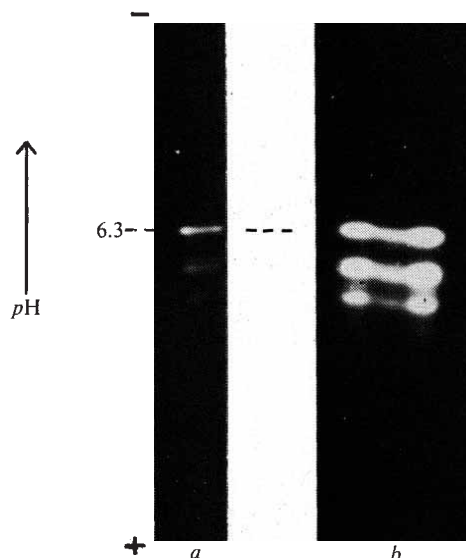


Fig. 1 Isoelectric spectrum of the anti-DNP IgG, produced by clone E9. The same characteristic spectrum was seen in the sera of recipient mice at each generation. a, Third transplant generation; b, seventh transplant generation.

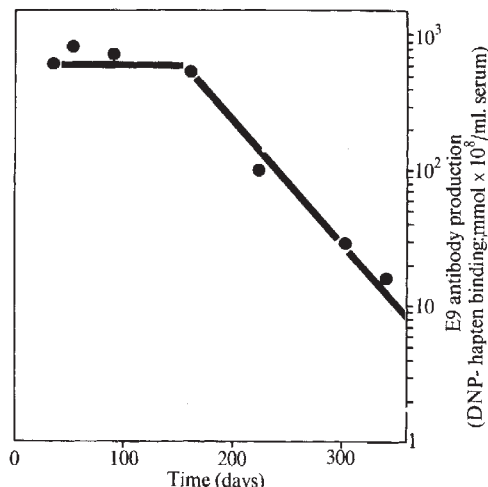


Fig. 2 Maximum production of E9 antibody in each transplant generation plotted as a function of the age of the clone. Each point represents the time at which the highest producer in each generation was killed for transplantation of clone E9.

transfer generation, plotted against the time at which that animal was killed for serial transfer of clone E9; the striking feature is the relatively constant, high production of E9 antibody through the first four generations of transplant recipients. Thereafter, maximum production declined successively in generations five, six and seven. The most obvious factor which could lead to this declining productivity would be a decreased number of antibody-forming cells. A less probable explanation is a depressed antibody production by the cells, but previous work has shown that antibody production is reasonably constant⁵⁻⁷.

It seems reasonable to assume that the level of E9 production in each recipient is directly proportional to the numbers of antibody-secreting cells. We can distinguish between two antigen-dependent processes controlling the number of antibody-secreting cells (Fig. 3): (i) the regeneration of memory cells by proliferation (n_2 divisions); (ii) the number of differentiated antibody-secreting progeny (burst size, n_3) arising from one memory cell on specific stimulation. The first is a purely proliferative event, but the second involves both proliferation and differentiation into, presumably, terminal antibody-secreting cells. The regeneration of memory cells is taken to be the major limiting factor in antibody production.

Evidence from many systems implicates antigen-specific thymus-derived cells (T-cells) in stimulation of antibody production by "bursa"-derived cells (B-cells)⁸⁻¹⁰. Stimulation of the memory cells (B-cell type) of clone E9 was shown to be specific with respect to the carrier protein on which the DNP groups were presented². This carrier specificity has been shown to be due to a requirement for antigen-specific T-cells acting together with antigen in eliciting antibody production by B-cells of clone E9 (ref. 1).

The proliferative failure of E9 memory cells in later generations could not be ascribed to a lack of T-cells capable of helping the B-cell response. At the fifth and sixth generation, extra T-cells were introduced in the form of spleen cells from mice primed with the carrier protein (BGG). Carrier primed cells almost doubled the mean E9 antibody response of six E9 recipients compared with the control group (Table 1). The highest response in the control group, however, was equal to the highest level of E9 antibody produced in the recipients given extra T-cells. By plotting maximum production in Fig. 2, we assume that any decrease in E9 production is not due to T-cell limitation. All recipients in the seventh generation received extra BGG primed cells.

The persistence of clone E9 in individual recipients is discussed elsewhere³. E9 antibody production has continued

Table 1 Level of E9 Production in Sixth Generation Recipients

Cells transferred	DNP-BGG	(Hapten binding capacity, mmol $\times 10^8$ /ml. serum)	Geometric mean
E9 spleen cells	With cells	24, 16, 7, 12, 4.8, 6, 6	9.1
E9 spleen cells plus BGG primed spleen cells	With cells	25, 12, 16, 19, 12, 24	16.2

Hapten binding capacity was measured by a modified Farr technique⁴ using 10^{-8} M α -N-(3:5-diiodo-4-hydroxy-phenacetyl)-N-(2:4-dinitro-phenyl)-L-lysine. In this system a titre of 100 represents about 500 μ g antibody/ml. serum.

over periods of about 6 months with antigen given intermittently. Regeneration of new memory cells is probably responsible for this persistence. We cannot assess the proliferation of memory cells in these conditions, where various homeostatic controls pertain.

The behaviour of clone E9 during successive transfer *in vivo* bore a striking resemblance to that of cloned human somatic diploid cell cultures passaged *in vitro*^{11,13}: both cloned populations exhibited a limited life span. Fibroblast-like cell strains from human foetal tissue underwent active division through 50 ± 10 serial passages ($2:1$ subculture)¹³, and this figure can be taken as a minimum estimate of the number of cell doublings during the life of the culture. A more realistic estimate, on the suggested basis of 3.5 doublings per passage¹¹, would be about 90 cell doublings before the end of the mitotic phase. Subsequent mitotic activity declines and cell generation time increases. These events have been interpreted as a senescence phenomenon at the cellular level.

The original amplification of clone E9 and its vigorous propagation *in vivo* through the first four transplant generations can be considered analogous to the phase of active mitosis in cell cultures. The ensuing steady decline in production of E9 antibody, which we have equated with a failure to regenerate memory cells, parallels the senescent phase of cell strains *in vitro*. The number of divisions undergone by cells of clone E9 during its lifetime *in vivo* can be estimated only approximately and with the aid of a number of assumptions.

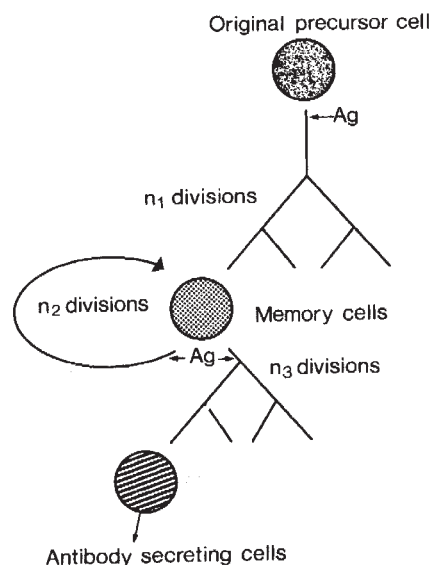


Fig. 3 Model of the development of an antibody-forming cell clone. The clonal precursor cell carries receptors recognizing antigen. Antigen-dependent divisions: n_1 , generation of memory cells from precursor cell; n_2 , regeneration of memory cells on further antigenic stimulation; n_3 , division and differentiation leading to terminal antibody secreting cells.

Clone E9 is not made up of cells at a uniform stage of differentiation, as is the case with fibroblast-like cell cultures. A simplified model of the growth of a clone is shown in Fig. 3. Our data suggest that it is the proliferative potential of memory cells for regeneration which controls the life span of the clone. It has not been possible to quantitate memory cells, but the number of antibody secreting cells (plaque forming cells) has been estimated at transfer generation 6 (A. J. Cunningham, unpublished results), and could be correlated with the serum antibody levels. The amplification of clone E9 in the first few transfer generations must have reached about 300,000 PFC in each mouse to account for the amounts of E9 antibody formed. From the work of Cunningham¹² and Sado *et al.*¹⁴, an approximate value of 10 divisions can be assigned to n_3 , the proliferative differentiation from memory cells into plaque forming cells. Thus several hundred memory cells must be reaching each host spleen. To generate sufficient memory for each subsequent generation to be distributed in about 10 hosts, and allowing for only 4% of viable memory cells reaching the host spleen¹⁵, it is necessary to make n_2 of around the same order of magnitude as n_3 at each generation. For the original amplification of the selected clone in the first generation n_2 must be about 20 divisions. The life span of clone E9 from its selection to a secreting cell in generation 7, or a memory cell for generation 8, would be not greater than 80 divisions. To this number must be added n_1 , which is presumably less than 10 divisions undergone between first antigen exposure of the differentiated precursor cell in the original donor and the selection of the clone by transfer.

Recent studies on the serial propagation *in vivo* of mouse mammary epithelium cells have shown that there is a decline in growth rate related primarily to the number of aggregate cell divisions. It was not possible, however, to estimate the division potential of these mammary cells¹⁷.

Our data are consistent with the idea that, with increasing differentiation, cells lose proliferative potential. From the first differentiation of the stem cell committing it to be a progenitor of an antibody-forming cell clone, the overall proliferative potential is not more than 90 divisions. This value is remarkably consistent with the life span of cloned somatic diploid cells *in vitro*. At any stage during the life span of a clone, cells may be recruited by antigenic stimulation into the antibody-secreting population. This further differentiation is apparently terminal in the sense that the proliferative potential of antibody-secreting cells is usually limited to a very small number of divisions¹². There is no evidence for the de-differentiation of antibody-secreting cells¹⁶.

The picture which emerges is that clonal senescence is a function of a finite proliferative potential of differentiated cells. This system is the first in which the senescence of a clone of cells has been followed *in vivo* and it has been possible to estimate the division potential of the clone.

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Received December 15, 1971;

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Dominance of a Cell Clone Forming Antibody to DNP

IN the previous paper¹ we described the history of a cell clone (E9) synthesizing a single species of anti-DNP. The original expansion of the clone and its strong proliferative capacity during the first few transfer generations were reflected by high levels of E9 anti-DNP production (2–3 mg/ml. serum). Throughout this period there was a striking absence of antibody to DNP other than the product of the E9 clone. The exclusive production of E9 anti-DNP was observed both through seven serial transfer generations, and when clone E9 was maintained in individual hosts for protracted periods of time with occasional reinjection of the original antigen (DNP-BGG).

At first, our strong selection for the E9 anti-DNP clone in host reconstituted with relatively few spleen cells (usually $3-5 \times 10^6$ cells²) might have excluded precursor cells forming other anti-DNP; however, with time, up to 7 serial transplants over a period of 8–10 months, or a period of up to 3 months within a single mouse, radiation recovery might be expected to result in the appearance of DNP-reactive precursor cells. In addition, after the fourth serial transfer, we added normal, or carrier protein (BGG)-primed, spleen cells, to replace any possible deficiency of T-derived helper lymphocytes, or other stem cells which could have been diluted out. Thus, the development or establishment of any other clones forming anti-DNP was, apparently, suppressed by the presence of clone E9 producing its characteristic antibody. This "dominance" of clone E9 held true so long as production of anti-DNP gave serum concentrations of above 50 μ g antibody/ml. With successive transplantation, the decreasing proliferative potential of clone E9 was indicated by progressively lower E9 anti-DNP levels in serum. Recipients of E9 producing less than 50 μ g E9 antibody/ml. serum produced a heterogeneous anti-DNP response after a second challenge with DNP-BGG.

Fig. 1A illustrates the clonal dominance of E9 anti-DNP at the third transfer generation.

Cell transfer took place 64 days after the original transplant of spleen cells primed with DNP-BGG into mouse E9. Spleen cells (4×10^6) from donors actively producing E9 antibody were transferred with 10 μ g DNP-BGG into a series of syngeneic irradiated mice. The isoelectric spectrum of serum anti-DNP of two host mice (numbered 5 and 9) is shown at three time intervals. *a*, 10 days after a further injection of 10 μ g DNP-BGG (36 days after cell transfer) *b* and *c*, 96 days after the cell transfer (76 days after the last injection of DNP-BGG). At all these times, the antibody has the same isoelectric characteristics, and no other anti-DNP antibodies could be found. After a further dose of 1 μ g DNP-BGG on day 96, exclusive production of E9 anti-DNP continued. The actual antibody titres at the different bleeding dates are given in Table 1.

Although the anti-DNP titres dropped considerably as the proliferative capacity of the clone declined¹, recipients of spleen cells containing the E9 clone continued to produce

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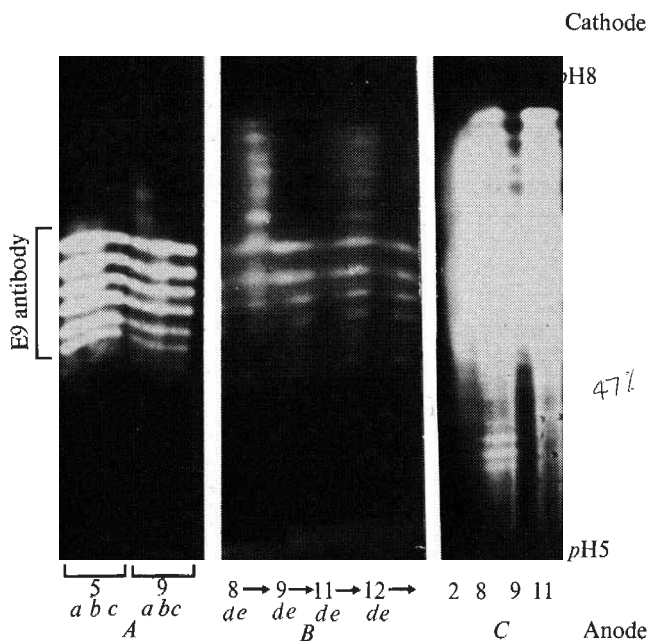


Fig. 1 Persistence of E9 antibody analysed by isoelectric focusing and specific detection of anti-DNP molecules with ^{125}I -labelled hapten and autoradiography³. **A**, Transplant generation 3; transfer of spleen cells from a second generation donor, together with 10 μg DNP-BGG on day 64 after selection of clone E9. Isoelectric spectra of E9 anti-DNP in sera of two recipient mice (numbers 5, 9). *a*, Bled day 75; *b*, bled day 100 (10 days after boost with 10 μg DNP-BGG); *c*, bled day 162 (96 days after cell transfers to third generation recipients). Serum anti-DNP titres are shown in Table 1. **B**, Transplant generation 6; cell transfer from fifth generation donor with additional BGG primed spleen cells and 1 μg DNP-BGG on day 251. Isoelectric spectra of anti-DNP in sera of individual mice (8, 9, 11, 12). *d*, Day 261 anti-DNP-titre, geometric mean=4.2; *e*, day 327 (10 days after boost with 1 μg DNP-BGG), geometric mean=6.7. **C**, Transplant generation 8; transfer from seventh generation donor with 1 μg DNP-BGG and BGG-primed spleen cells on day 323. Isoelectric spectra of anti-DNP in mice 2, 8, 9, 11, on day 365 (15 days after boost with 1.5 μg DNP-BGG).

exclusively E9 anti-DNP for several more months, as long as the hapten-binding capacity exceeded 10 mM hapten $\times 10^{-8}$ /ml. serum (or about 50 μg E9 antibody per ml. serum). Fig. 1B shows the isoelectric spectra of sera of host mice receiving E9 spleen plus antigen eight months after the original E9 cell transfer (transplant generation 6). Ten days afterwards, only E9 anti-DNP could be detected in the serum of these recipients; the antigen-binding capacity of the sera had dropped to 4.2 (geometric mean of 8 sera). The mice received a boost injection of antigen (DNP-BGG) on day 38, and on day 48 the sera showed at least 2 additional anti-DNP clones (Fig. 1B, *e* series). By transplant generation 8 (11.5 months after appearance of the E9 clone) DNP-BGG induced a heterogeneous anti-DNP response (Fig. 1C).

Table 1 Persistence of E9 Production in Third Generation Recipients

	Hapten binding capacity (mmol $\times 10^8$ /ml. serum)		
	<i>a</i> (post cell transfer)	<i>b</i> (10 days post boost)	<i>c</i>
Mouse 5	88	130	48
Mouse 9	150	234	140

10 μg DNP-BGG plus 4×10^6 spleen cells from a second generation E9 donor were transferred intravenously into syngeneic, irradiated (660R) mice on day 0. Hapten binding capacity was measured as previously described¹. Day 25 boost intravenously with 10 μg DNP-BGG.

These examples have been chosen from extensive data on clone E9. A number of conclusions can be drawn from consideration of the history of clone E9, which will be published elsewhere in detail¹⁷. E9 antibody does not shut off memory cells belonging to its own clone; it has been possible to transfer antigen responsive memory cells of clone E9 for up to 2-3 months after the previous cell transfer into the donor, although continued production of E9 anti-DNP has taken place meanwhile. The predominance of E9 anti-DNP to the exclusion of other antibodies to DNP was maintained over a period of 8-10 months and seven transfer generations, as well as for 3 months within an individual mouse. The presence of E9 antibody (K_a about 10^7 l. mol^{-1})⁵ does therefore appear to suppress the establishment or development of other cell clones reactive to DNP. It has been shown earlier that administration of 7S antibody inhibits the induction of antibody against the same antigen. A secondary response is less sensitive to suppression by passive antibody than is the response to first injection of antigen⁶. Although the mechanism of antibody suppression is not clear, and possibly varies under different experimental conditions, a number of possible explanations can be considered.

Competition for antigen can occur between immunoglobulin cell receptors on memory cells and circulating antibody with the same specificities. The fact that E9 antibody does not turn off production of E9 anti-DNP suggests that this control may only be effective for memory cell receptors with lower affinity than the circulating antibody. This mechanism would thus select for high affinity clones, and it has been observed that the affinity of antibody increases with time of immunization⁷. Our observation that no clones with higher affinity anti-DNP than E9 have established themselves until the E9 antibody level dropped below 50 μg anti-DNP/ml. would suggest that this cannot be the complete explanation.

Immature antigen-reactive precursor cells may be more sensitive to the presence of antibody competing for antigen than are more mature memory cells; this could be due to a lower receptor density on immature cells. The presence of antibody alters the overall handling of antigen and the mechanism of antigen presentation to reactive lymphoid cells. In other clonal selection experiments, animals bearing two clones have been examined; the clones could be transferred together, which suggests that memory cells of other antigen-expanded clones are not suppressed as readily as immature precursor cells which have not previously seen antigen.

Antibody has also been shown to facilitate tolerance induction in immature cells⁸. As, in our case, however, clonal dominance is broken at low levels of E9 anti-DNP production, there is no evidence that tolerance plays a part.

The long life of antibody-forming cell clones correlates with the persistent production of antibody carrying a given idiotypic determinant observed in rabbits immunized during several months^{9,10}. Monoclonal anti-DNP antibody production, elicited in rabbits by di-DNP-gramicidin, has been found to continue for many months⁵. Clonal dominance is probably responsible for the restricted antibody response resulting from hyperimmunization of rabbits with pneumococcal¹¹ or streptococcal vaccines¹², or with hapten-protein conjugates^{13,14}. Certain rabbits mounting high responses show restricted or even monoclonal antibody, suggesting that a single clone of antibody-forming cells has taken over and suppressed the development of other antibody-forming cell clones¹⁵. It might be argued that since bone marrow recovery in the irradiated recipients at each transfer takes several weeks¹⁶, there would not have been sufficient opportunity for diverse new cell lines producing anti-DNP to develop. In those transfers, however, when carrier (BGG) primed or normal unprimed spleen cells were added, some other anti-DNP precursor cells would almost certainly have been included. In fact, their presence is attested by the fact that when E9 antibody levels dropped below 50 μg /ml. serum, a heterogeneous anti-DNP response resulted.

In summary, cell clone E9 dominated the anti-DNP response while the clone was actively proliferating and producing antibody. This exclusive production of E9 anti-DNP, with its characteristic isoelectric spectrum, persisted for up to 100 days within individual mice, and through several serial spleen cell transfers. The implication is that immature anti-DNP precursor cells are not able to proliferate or differentiate in the presence of clone E9 forming large amounts of antibody. Clonal dominance was complete as long as E9 production was in excess of 50 µg anti-DNP per ml. serum.

We thank B. E. G. Wright for his assistance.

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Received December 15, 1971.

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Sporozoite and Normal Salivary Gland Induced Immunity in Malaria

STUDIES using sporozoites as antigenic material to induce protection against malaria have been relatively scarce compared with those employing antigens of the blood stages. Many workers¹⁻⁵ have reported considerable protection against challenging homologous sporozoites of *Plasmodium gallinaceum*. Richards⁴, using three injections of sporozoites exposed to ultraviolet, dried or treated with formalin, found at least eighteen birds out of twenty were protected, but with freeze-thawed sporozoite material he found only fifteen out of twenty protected. By using six to seven injections of X-irradiated sporozoites of *Plasmodium berghei*, Nussenzweig *et al.*^{6,7} obtained 90–100% protection when the animals were challenged with 2,000 sporozoites two weeks after the last injection.

In the experiments described here we used 3–4 week old A/J inbred female mice which were randomly grouped according to weight. All animals were vaccinated intraperitoneally with *Plasmodium berghei* NK65 and challenged intraperitoneally with 2,000 sporozoites. Those which had positive blood films died.

In the first experiment, seven injections of 25,000 sporozoites, heat inactivated at 40°–42° C for 45 min, were given at intervals of fourteen days. After challenge, seven out of eight normal control mice became infected while four out of eight control mice injected with salivary gland protein and nine out of ten sporozoite injected mice showed no infection.

In the second experiment, six injections of 450,000 sporozoites, heat inactivated at 40°–42° C for 60 min, were given

as in the first experiment. After challenge, all ten normal control mice became infected, while seven out of ten salivary gland control mice and seven out of nine sporozoite injected mice remained uninfected.

In the third experiment, six injections, each of 400–500 µg protein of normal salivary gland material heated at 40°–42° C for 60 min, were given at intervals of fourteen days. Following challenge at nineteen weeks, all the ten untreated control mice and six out of nine normal salivary gland mice became infected.

In the fourth experiment, six injections of 450,000 sporozoites, inactivated by freezing and thawing three times, were injected at fourteen day intervals. After challenge, none of the ten normal control mice remained uninfected, but seven out of ten salivary gland injected and nine out of ten sporozoite injected animals remained negative. Thus freezing and thawing do not seem to inhibit the activity of the antigen.

The most striking result in each of the experiments is that controls injected with tissue from normal mosquitoes showed unexpectedly good protection although always at a somewhat lower level than sporozoite injected mice. Nevertheless some protection appears to last as long as nineteen weeks, which is equivalent to about a fifth of the mouse life span. The simplest explanation for this protection seems to be non-specific macrophage type immunity, although Rivera⁸ reported that mouse non-specific spleen activity usually returns to normal fourteen days after similar injections. Our results suggest that the protection with normal salivary gland material is not of a non-specific macrophage type.

Alternative explanations for this protection may possibly be antigenic mimicry as suggested by Damian⁹ or the combination of antibody with mosquito antigen carried on the surface of the sporozoite which, with the aid of complement, may destroy the parasite. Alternatively there may be cross-reaction between mosquito and parasite antigens, or possibly antigens of contaminating bacteria or viruses cross-react with the parasite.

Neither heat treating nor freeze-thawing seems to interfere with the induction of protection by either sporozoite or normal salivary gland antigens.

Although the possibility of protective immunization with normal mosquito tissue is exciting because the various stages of the parasite itself are so difficult to procure, the mechanism of this immunity remains obscure. Fractionation of both sporozoite and normal mosquito tissue may be a first step toward understanding this phenomenon.

We thank Barbara Cole and Rose Ann Meccoli for help. This work was supported by a United States Agency for International Development contract.

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Estimation of Steam-Volatile N-Nitrosamines in Foods at the 1 µg/kg Level

SINCE the discovery¹ of the hepatotoxic and carcinogenic properties of N-nitrosodimethylamine (DMN), these properties have been demonstrated in a variety of N-nitroso compounds²⁻⁵. The effect of feeding mink with herring meal treated with excessive amounts of nitrite is to induce a liver disease⁶, and DMN has been isolated as the hepatotoxic factor^{7,8}. Various methylamines present in the herring meal, especially dimethylamine and trimethylamine, were found to be responsible for the formation of DMN. Although the concentration of nitrite used in the processing of the fish meal were not typical of normal commercial food preservation practice, it is essential that any potential nitrosamine hazard deriving from the use of nitrites and nitrates in the preserving and processing of foodstuffs should be evaluated.

The principal chemical route to N-nitrosamines is the reaction of secondary amines with nitrous acid in the presence of nitrite ion. Early studies on the kinetics of the reaction⁹ have been supported by recent work using radiochemical techniques¹⁰. Any assessment of a possible hazard due to the occurrence of nitrosamines in the food chain should take into account likely combinations of the necessary precursors and the conditions (for example, concentration, temperature and pH) under which such combinations can occur.

Sander *et al.*¹¹ have shown that it is possible for nitrosamines to be produced *in vivo* from precursors, ingested at relatively high levels. Nitrates are widely distributed throughout nature and are readily reduced to nitrite by bacterial action and they must be considered in any study. There is little information on the distribution of secondary amines in foods, but Lijinsky and Epstein¹² have postulated that they may be produced by pyrolysis of proteins in the cooking of food.

Sensitive and specific analytical methods for the identification and estimation of N-nitroso compounds in food products are essential for the evaluation of any health hazard due to N-nitrosamine formation. Many of the techniques employed in the early studies were of relatively poor sensitivity, and lacked discrimination. Thus the results of analysis of extracts from foodstuffs after clean-up by column chromatography using ion-exchange resins, alumina or charcoal, employing methods such as thin-layer chromatography¹³, gas-liquid chromatography¹⁴, polarography¹⁵ or nitrite-release¹⁶, can be ambiguous. Gas-liquid chromatography and mass spectrometry provide more reliable characterization of individual N-nitrosamines. Telling *et al.*¹⁷ reported lower limits of detection varying from 25 µg/kg to 65 µg/kg for a range of volatile nitrosamines by monitoring the common fragment ion NO⁺. Gough and Webb¹⁸ reported detection limits of 2 µg/kg by monitoring the appropriate parent ions lowered to 0.2 µg/kg by the use of peak cutting techniques. In all cases reported preparation of a suitable extract gave a concentration factor of about 1,000.

Various methods of separation of volatile N-nitrosamines and clean-up of extracts have been investigated in these laboratories¹⁹. In this study we have utilized two types of nitrogen selective detector for preliminary examination of food extracts, and where an apparently positive response was obtained the extract was examined further by the combined GLC-mass spectrometry technique of molecular ion monitoring using a high resolution instrument¹⁸. We describe a method for the detection, identification and estimation of steam-volatile N-nitrosamines in foods down to 1 part in 10⁹ (that is, µg/kg). Initial findings in a restricted survey of certain varieties of foods including bacon, fish and cheese are reported. The effects of conventional cooking methods have also been studied and are reported. Compounds studied were the N-nitroso derivatives of dimethylamine (DMN), methylethylamine (MEN), diethylamine (DEN), dipropylamine (DPN), dibutylamine (DBN), pyrrolidine (PYR) and piperidine (PIP).

Varian 1200 and 1740 gas chromatographs were used. The 1200 instrument was fitted with a 5.5 m × 3 mm i.d. stainless steel column packed with a 15% FFAP on 'Chromosorb W' (80-100 mesh). The outlet from the column was connected via a heated transfer line to a 'Coulson Conductivity Detector' operating in the reductive mode for nitrogen. The 1740 instrument was fitted with a 5.5 m × 3 mm i.d. stainless steel column packed with 15% 'Carbowax 20M' on 'Chromosorb W' (80-100 mesh). The modified flame thermionic detector (rubidium sulphate tip) gave the optimum response to standard solutions of N-nitrosamines.

For the mass spectrometry an 'AEI MS902' high resolution instrument was used. The sample was injected into a 'Pye (model R)' gas chromatograph fitted with a 5.5 m FFAP column. The effluent stream from the column was split in a single stage membrane separator, part going by a heated stainless steel transfer line to the mass spectrometer and part into a standard FID detector. Full details of the technique used have been described by Gough and Webb¹⁸.

The sample was minced and 250 g, with 54 g NaCl, was steam distilled; 350 ml. of distillate was collected. 6 ml. of 6 N sulphuric acid and 42 g sodium sulphate (anhydrous) were added to the distillate and the resulting solution was redistilled, 250 ml. being collected. About 0.2 g of solid potassium hydroxide and 50 g sodium chloride were added to this distillate which was then extracted three times with 50 ml. portions of methylene chloride. The organic layers were combined, dried over sodium sulphate, and evaporated in a Danish-Kuderna apparatus at 50° C, until approximately 2.5 ml. of solvent remained. 1 ml. of n-hexane was added and the evaporation was continued until 0.25 ml. remained. The concentrated extract was examined by gas chromatography and the results confirmed where necessary by mass spectrometry.

We initially studied those foods likely to contain the essential precursors for nitrosamine formation; these are cured and processed meats including bacon, ham and salami; cured and

Table 1 Volatile Nitrosamines and Nitrite Levels in Fried Bacon

Description	N-Nitrosamines* µg/kg (uncorrected)				Nitrite content of original sample mg/kg
	DMN	DEN	PYR	PIP	
Back rashers, prepacked	T	ND	A	ND	—
Back rashers, prepacked meat only	T	ND	T	ND	—
Back rashers, prepacked fat	T	ND	T	ND	—
Back rashers, Danish smoked	ND	ND	T	T	—
Collar, Irish	T	ND	ND	ND	—
Middle, Ayrshire, pre- packed	T	ND	T	ND	—
Back, smoked, Danish	T	T	ND	ND	50
Back, pale, Danish	A	ND	C	ND	20
Gammon, pale, Danish	A	ND	ND	ND	30
Gammon, smoked, Danish	A	ND	ND	ND	40
Back, pale	A	ND	B	ND	5
Back, smoked	A	ND	A	ND	2
Gammon, smoked	ND	ND	A	ND	30
Gammon, smoked	ND	ND	ND	ND	5
Back, pale	ND	ND	A	ND	10
Back, smoked	A	ND	ND	ND	40
Gammon, pale	ND	ND	ND	ND	10
Gammon, pale	ND	ND	ND	ND	—
Foreback, pale	ND	ND	ND	ND	5
Foreback, pale	ND	ND	A	ND	5
Foreback, pale, Danish	T	ND	ND	ND	20
Forehock, smoked	ND	ND	A	ND	20
Forehock, smoked	ND	ND	ND	ND	60
Forehock, smoked, Danish	ND	ND	A	ND	—

* ND = Not detected. T < 1 µg/kg. A = 1-4 µg/kg. B = 5-9 µg/kg. C = 16-40 µg/kg.

fresh fish; and a range of cheeses, especially those varieties in which the use of nitrate or nitrite is permitted. The results are presented in Tables 1, 2 and 3. The concentrations quoted in the tables were obtained by measurements of the intensities of the molecular ions using the mass spectrometer and at these very low levels are subject to an error of $\pm 50\%$, and are shown as concentration ranges. In most cases parallel measurements with both GC detectors produced results of a similar order, but for extracts from samples of some cooked foods interference from other nitrogenous compounds occurred which made quantitative measurements of the lower molecular weight species difficult. This was especially true of fried samples. A number of recovery trials have been carried out adding to samples of both the raw and cooked food a mixture of all six volatile N-nitrosamines studied at the 10 $\mu\text{g/kg}$ level. Overall recoveries have been variable but normally fell within the range 70 to 90%. N-nitroso pyrrolidine is exceptional in that very low recoveries were obtained at the initial steam distillation stage and overall recoveries were generally below 30%. No correction factor to allow for losses sustained during the analytical method has been applied to the concentration ranges quoted in the tables.

Table 2 Volatile Nitrosamine and Nitrite Levels in Fish

Description	DMN $\mu\text{g/kg}$ *	Nitrite in original sample mg/kg
Kippers	ND	Tr
Kippers, fried	ND	Tr
Salmon, smoked	ND	—
Haddock, smoked	ND	—
Herring, pickled	ND	—
Cod, salted	A	—
Cod, salted	A	Tr
Cod, fresh	A	Tr
Skate, fresh	ND	Tr
Mackerel, fresh	ND	Tr
Plaice, fresh	ND	Tr
Cod, steak in breadcrumbs, fried	A	Tr
Cod, fillet, fresh	ND	—
Cod, fillet, fried	A	—
Hake, fillet, frozen	ND	—
Hake, fillet, fried	A	—
Cod, steak in batter	ND	Tr
Cod, steak in batter, fried	ND	Tr
Hake in breadcrumbs	ND	Tr
Hake, steak, fresh	A	—
Hake, steak, fried	B	—
Hake, fresh	ND	—
Cod, fresh	ND	—
Cod, fried	A	—
Cod, fresh	ND	—
Cod, fried	A	—
Haddock, fresh	ND	—
Cod, fresh	ND	—
Cod, fried	A	—
Haddock, fresh	ND	—
Haddock, fried	A	—
Haddock, baked	B	—
Cod, fresh	A	—
Cod, fried	B	—
Coley, fresh	ND	—

* ND=Not detected. A=1–4 $\mu\text{g/kg}$. B=5–9 $\mu\text{g/kg}$. Tr=2 mg/kg nitrite.

So far only a relatively small number of samples purchased at random has been examined and many of these were of unknown processing history. They may not be typical of the commodities as a whole. Despite these limitations the picture which emerges is that minute quantities of certain N-nitroso compounds which are known to be biologically active at higher levels in animals have been detected in some foods. The available information does not yet permit any definite conclusion concerning the biological effects for man of minute amounts of N-nitrosamines in foods. Meanwhile further analytical information on fish, cheeses, cheese products, meats

Table 3 Volatile Nitrosamine Levels in Miscellaneous Food Products

Description	Volatile nitrosamines $\mu\text{g/kg}$ *		Nitrite mg/kg
	DMN	DEN	
Spinach, chopped leaf in brine	ND	ND	—
Frying oil (used)	ND	ND	—
Luncheon meat	A	ND	3
Pork, chopped, Danish	A	A	—
Salami, Austrian	ND	ND	2
Salami, Polish	ND	ND	4
Salami, Kosher	ND	ND	380
Salami, German	ND	ND	<2
Salami, Hungarian	A	ND	4
Salami, Italian	ND	ND	<2
Cheese, Cheddar, matured	ND	ND	<2
Cheese, Grinland	A	ND	<2
Cheese, Danbo	ND	ND	—
Cheese, Edam	ND	ND	—
Cheese, Harvant	ND	ND	<2
Cheese, Gouda	ND	ND	<2
Cheese, Danish Blue	A	ND	<2
Cheese, Gouda matured	A	ND	<2
Cheese, St Paulin	A	ND	<2
Cheese, Norwegian Tilsit	A	ND	<2
Cheese, Norwegian goats' milk	A	ND	<2
Cheese, Stilton	ND	ND	—
Bacon, Irish back	ND	ND	—
Ham, smoked, Westphalian	ND	ND	30
Hamburger, minced beef	ND	ND	—

* ND=Not detected. A=1–4 $\mu\text{g/kg}$.

and meat products is required and additional studies have commenced.

We thank our colleagues for technical assistance. The work was supported by the Ministry of Agriculture, Fisheries and Food, and the Department of Health and Social Security.

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Received February 28, 1972.

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IgM-IgG1, 2, 3 Relationship during Pregnancy

In mice, paternal tissue grafts generally survive longer in the mother with each succeeding pregnancy¹. Similar findings have been reported for rats, rabbits and humans². Paternal tumours also survive longer in pregnant mice³. However, mating across strain does include cellular immunity directed against paternal antigens, measured by graft versus host activity⁴ and *in vitro* killing of hybrid foetal cells⁵ by maternal lymphocytes. Thus the survival of experimental allografts^{1,3} and of the natural foetal "allograft" in the face of maternal cellular immunity may depend on immunological enhancement, that is, the protection of foreign grafts by specific antibody which blocks cellular immune rejection^{3,5}.

Serum antibody responses to several heteroantigens in mice, hamsters, and guinea-pigs are not grossly altered by pregnancy⁶, nor is the response of rabbits to an isoantigen on rabbit red cells⁷. However, these studies would not have revealed a preferential stimulation of antibodies in a minor class that could block complement fixing antibody¹⁵ (IgM, IgG2) and sensitized lymphocytes⁵ from killing foetal or placental tissues. Of particular interest is the newly described IgG3 class of mouse immunoglobulin which does not fix complement and which is preferentially transported to the foetus^{8,19}. We find that IgG3 antibody synthesis is stimulated during pregnancy, but also IgG1 and IgG2. IgM antibody synthesis is decreased.

Spleen cells from mice immunized with sheep erythrocytes were assayed for IgM antibody synthesis by direct plaque

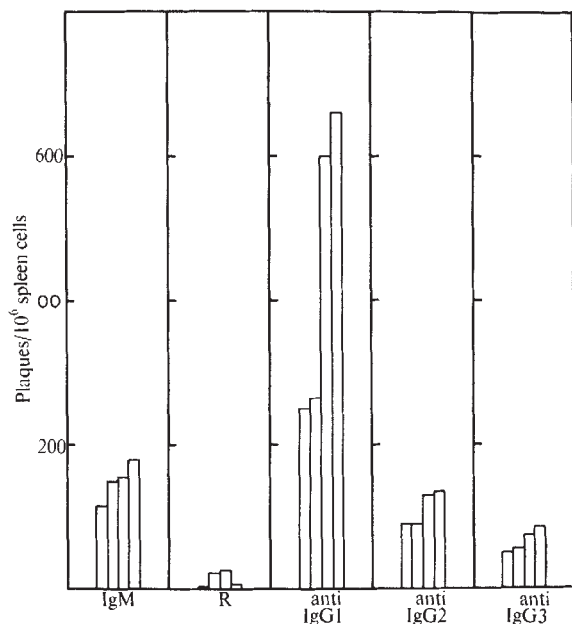


Fig. 1 Four female BALB/c mice 3–6 months old were immunized i.v. with 0.2 ml. of 10% v/v suspension of sheep red cells. Spleens were assayed separately seven days later for plaque forming cells⁹. IgM plaques were measured as direct plaques: spleen cells were incubated with sheep cells in agar 1 h at 37° C, then in guinea-pig complement 1:10 for 2 h at 37° C. Duplicate slides were reduced and alkylated¹⁰ before incubation in complement, to inhibit the formation of IgM plaques (R). (Reduction did not appear to affect the numbers of IgG1 p.f.c. in the primary response at day 7, or of IgG1, 2, or 3 p.f.c. in the secondary response at day 6; unpublished observations.) Other slides were similarly reduced and incubated in complement together with an optimal dilution (1:400) of absorbed, class specific rabbit anti-(mouse immunoglobulin) antisera (see text and Fig. 2). Each rabbit antiserum was freed of cross-reacting antibody activity by passage through immuno-adsorbent columns of 'Sepharose' coupled with myeloma proteins of the other immunoglobulin classes¹¹. Each bar of a group represents the number of plaques per 10⁶ viable, nucleated spleen cells for one animal. Duplicate slides were made at several spleen cell concentrations.

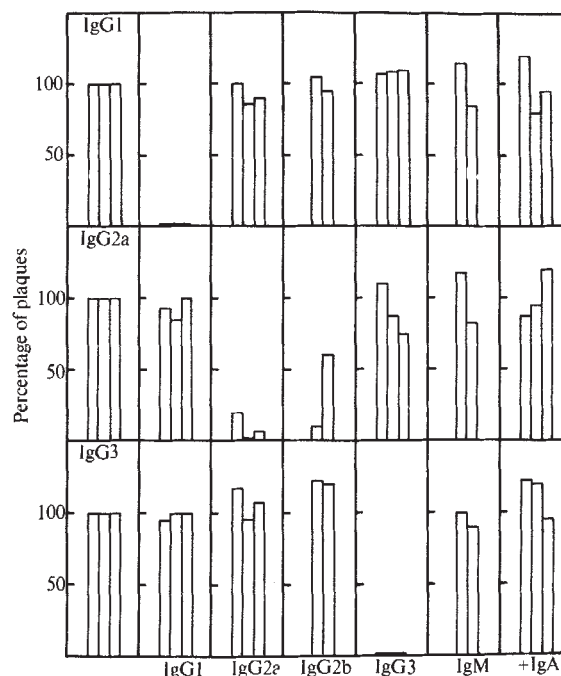


Fig. 2 Plaque forming cells were assayed as described in the legend to Fig. 1. All slides were reduced to inhibit IgM plaques. The small numbers of reduction resistant plaques were subtracted from the plaques seen with rabbit amplifying sera present in the complement solution. The first column represents the plaques developed with anti-IgG1, 2, or 3 antiserum as shown. To demonstrate the specificity of each serum, duplicate slides were incubated in complement to which purified myeloma proteins of the different mouse immunoglobulin classes had been added, together with the specific antiserum. The amount of purified myeloma protein which just inhibited each homologous antiserum was about 1 µg/ml., and 2 µg/ml. was used in these tests. The results are normalized to the values of the first column for which no myeloma protein inhibitors were present.

formation (Fig. 1). On duplicate slides, IgM plaques were inhibited to less than 10% by reduction (Fig. 1, R). Cells making antibody of the IgG1, 2, or 3 classes of immunoglobulin were determined by subsequently developing the slides in complement solution containing rabbit "amplifying" antiserum specific for mouse IgG1, 2, or 3, respectively (see Fig. 1). Few IgA cells were observed and will not be considered in this paper. The peak of IgM plaque forming cells (p.f.c.) occurs at day four to five after immunization (ref. 10 and unpublished observations), when it is difficult to measure the smaller number of IgG cells. Day seven was therefore chosen because IgM cells do not seriously interfere with the study of IgG p.f.c.

The specificity of each amplifying antiserum in developing plaques of only one immunoglobulin class was tested by inhibition of plaque formation, by addition of myeloma proteins. As shown in Fig. 2, the development of IgG1 plaques by rabbit anti-IgG1 was completely inhibited by the presence of IgG1 myeloma protein, but not by IgG2a, IgG2b, IgG3, IgA or IgM myeloma proteins. Similarly, IgG2a inhibited most of the IgG2a plaques and IgG3 inhibited all of the IgG3 plaques whereas myeloma proteins of the other classes had little effect. The one exception was IgG2b protein which partially inhibited IgG2a plaques, indicating some remaining cross-reactivity in this amplifying antiserum.

IgG3 immunoglobulin in maternal BALB/c sera represents about 9% of all IgG classes⁸. Spleen cells with IgG3 immunoglobulin on their surface constitute about 3% of IgG positive cells¹². We find that 12% of all IgG p.f.c. at day 7 after immunization are IgG3 cells (Fig. 1). This represents the first demonstration of antibody activity in the IgG3 class, apart from the myeloma protein J606⁸.

Fewer spleen cells forming IgM antibodies were found in

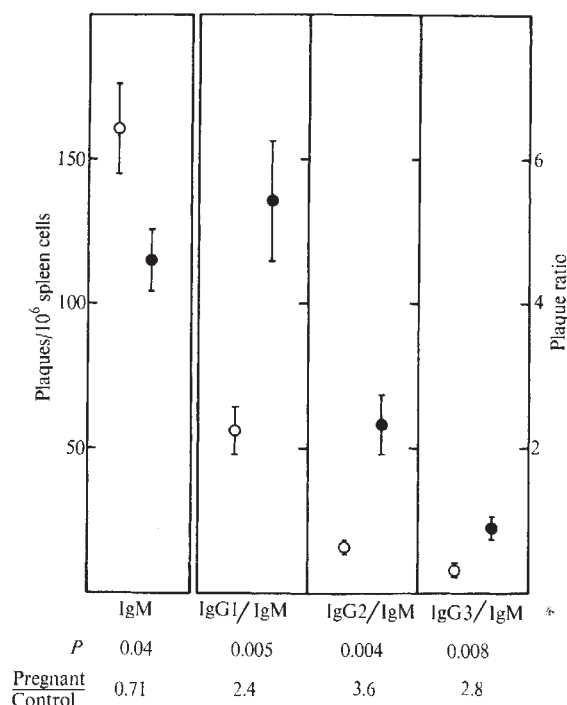


Fig. 3 Ten BALB/cJ mice at 13–14 days of pregnancy (●), timed by finding a plug at day 1 (Jackson Laboratory), and eleven control female BALB/cJ mice (○) were immunized with sheep red cells. Mice were 3–6 months old. Spleens were assayed for plaque forming cells seven days later as described in the legend to Fig. 1. Similar numbers of spleen cells were recovered from both groups. Mean values of direct (IgM) plaques and of the ratios of indirect to direct plaques are shown for each group $\pm$ standard error. Half of the pregnant mice delivered on the morning of the experiment; they did not differ significantly from the gravid mice except that they had fewer IgG2 plaques. *P*, The statistical significance of the differences between control and pregnant mice was calculated by Cochran's modification of Student's *t* test²⁰.

pregnant mice compared with controls (Fig. 3). In contrast, p.f.c. of all three IgG classes were more numerous in the pregnant group. Relative to IgM p.f.c., IgG1, 2 and 3 cells were 2.4 to 3.6 times as frequent in the pregnant mice compared with the controls. This increase in IgG and decrease in IgM p.f.c. may occur uniformly throughout the immune response, or may reflect an alteration in the transition from IgM to IgG antibody synthesis. Preliminary experiments suggest that the earliest differences in pregnant mice are increases in IgG p.f.c. at day 4 and in serum IgG haemagglutinins at day 5 (first day detectable).

Normal pregnancy is initiated and maintained despite strong prior immunization of the female to paternal or foetal antigens¹³. The placenta is an efficient anatomical barrier to maternal lymphocytes and to highly cytotoxic IgM antibody¹⁴. IgG antibody of cytotoxic classes is transferred, however, from the maternal circulation to the foetus in mice, rats, rabbits and man¹⁴, and there is a functional complement system in the newborn of these species². Furthermore, the placenta and other extra-embryonic membranes are themselves directly exposed to maternal immune rejection.

IgG antibody with complement kills target cells only at high densities of surface antigenic determinants^{15,16}, and protects cells from cytotoxic IgM antibody at low densities¹⁵. Placental trophoblast¹⁷ and foetal cells¹⁸ express fewer histocompatibility antigens on their surface compared with adult cells, which may confer protection from mainly IgG complement-fixing antibody.

Protection from cell mediated immunity must also be considered, at least for placental cells. A secreted sialomucin is believed to play a protective role for the trophoblast¹⁷. Immunological enhancement, that is, blocking by IgG antibody,

would also protect foetal tissue from cell mediated immunity^{8,9}. The increase in IgG to IgM antibody reported in this paper may be a manifestation of enhancement. Synthesis of IgG3 antibody is increased during pregnancy, along with the other IgG classes. However, IgG3 is transported to the foetus 20–50 times more rapidly than IgG1 and IgG2 (see ref. 19), and represents 40% of the immunoglobulins in newborn mice⁸. Thus this non-complement fixing class may provide the foetus with passive immunity (antibodies to pathogenic organisms) while protecting against destructive maternal immune responses (blocking antibody).

It is likely that mammals utilize several devices—placental barrier, low density or masked surface antigens, blocking antibody, etc.—for overlapping protection during pregnancy. To explain the paradoxical survival of the foetus in the maternal immune environment, we need better knowledge of the antigenic density of foetal cells, of the kinds of anti-foetal antibody produced and transferred into the embryo, and of the quantitative relationships between IgG antibody, IgM antibody and sensitized lymphocytes in cell killing.

This research was supported by a grant from the Ford Foundation. We thank Dr H. Grey for a supply of antiserum to IgG3.

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Received January 21; revised April 13, 1972.

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Age, the Crystalline Lens of the Rudd and Visual Pigments

THE retinae of the rudd contain more of the visual pigment VP₁₅₀₇ in the summer, but more of VP₂₅₃₅ in the winter¹. The interconversion is shown to be governed by the local light environment² since it can be effected in one of the two eyes by occluding it. Thus, light must act directly, and not, for example, by some humoral process. No action spectrum of the process is available for this species (but see ref. 3), an essential for the understanding of such phenomena⁴, but the fact that only relatively young members of this species can achieve the conversion² is significant. Individuals seven years old or more keep VP₂₅₃₅ throughout the year. This

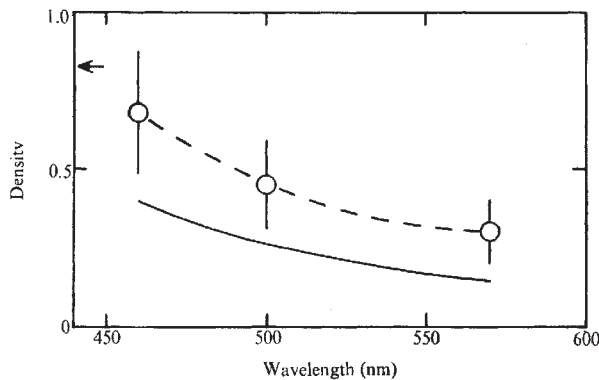


Fig. 1 Density of lenticular pigment (ordinate) against wavelength of maximum transmission of filter used (abscissa). $\circ$ --- $\circ$, Average results for old rudd; —, results for a 53-year-old human lens¹⁴. The arrow marks the log ratio of $I_M \delta t$ where I_M refers to the illuminance of the sky in June and December respectively and t is the time¹⁶.

"winter" pigment occurs in the retinæ of young fish only after about one month's stay in the dark, or if the corresponding corneae are occluded.

Although ageing fish may lose the ability to convert visual pigments², the simpler alternative is to assume that, for some reason, their retinæ—or pigment epithelia⁵—remain in relative darkness even though there is enough light for conversion to occur. The crystalline lenses of several vertebrate species are known to yellow and to darken with age^{6,7}; perhaps the inner darkness of the rudd retina is caused by just such a partial occlusion. The spectral transmissivity of fish lenses has been measured by several authors^{8–11}: their sphericity makes it difficult to obtain absolute values for the spectral transmissivity T , and no curve for the senile variation appears to have been published for the rudd¹².

We obtained two groups of fish of the same strain (*Scardinius erythrophthalmus*) from the same source as Dartnall *et al.*¹ and Bridges and Yoshikami², that is, the Surrey trout farm, and we thank Mr D. F. Leney for information on their ages. Group A was aged 3 years, group B 7 to 8 years; these bracketed the transition age above which the fish failed to transmute pigments. The average lens masses are given in Table 1. As fish size is no reliable guide to the age of the specimen, it cannot be assumed that lens mass is any more useful in this respect¹³. The colourless lenses¹² from group A had an optical density less than 0.2. This was also true of the outer layers of those from group B, but they surrounded a deeply yellow inner region. Going from the anterior surface of the lens it was located in the second quarter, sharply discontinuous on its anterior aspect but diffuse in the vitread direction. This was verified when the lenses were placed in saline, and also by compressing them between two parallel microscope slides. The transverse extent of the yellow-brown section approximately equalled the pupillary diameter. It did not seem to be a cataract. The transmissivity was estimated by illuminating the lens with light passed in turn through 'Ilford' Bright Spectrum Filters Nos. 621, 623 and 626, the lens being placed on a microscope slide at some distance above a white diffusing surface. The dark central patch was matched with calibrated neutral density filters occluding part of the unpigmented lens. Fig. 1, which gives the mean of the matches on 5 lenses, shows that this crude method can yield reasonably consistent results. The dashed curve¹⁴ shows that within one year or so, part of the colourless rudd lens has become optically

denser than does a thicker 53-year-old human one in several decades. The rudd lens pigment is not distributed uniformly throughout the lens, although this is the case in man¹⁵.

A senile increase in lenticular light absorption appears therefore to interfere with pigment conversion in the retina of the rudd. The action spectrum of the process may therefore peak at a wavelength shorter than 507 nm, that is, in a region where the intensity reaching the retina may be reduced by over five times³. The maximum of the spectral distribution of ordinary daylight¹⁶ lies at about 470 nm, and the southern English sky has a product (illuminance $\times$ time) of 6.7 times greater in June than in December¹⁶. The average correlated colour temperature changes between December and June from approximately 6,500 K to 9,000–25,000 K, with a dominant wavelength of about 470 nm¹⁷. Fig. 1 shows therefore that lenticular yellowing mimics the effects of the winter period. This view is reinforced by an apparent phase lag¹ of 1 month between the pigment interconversion cycle and the seasonal variation in day-length. The pigment conversion following artificial occlusion takes about four weeks to achieve, and this period may be the duration of life of the rod. If interconversions represent an adaptive phenomenon⁴, then the relative smallness of the differential threshold intensity cannot come as a surprise. As rudd spend their lives in fresh shallow water of reasonably constant spectral transmissivity there seems to be no variation in the environmental illumination to account for our findings.

To sum up, we propose that old age affects visual pigment transmutation in the rudd in the same way as the change from summer to winter, because the yellowing of a photically important part of the animal's crystalline lenses reduces its retinal illumination by a factor commensurate with the ratio of the integrated daylight illumination for June and December respectively.

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The Response of Human Pleura in Organ Culture to Asbestos

FOLLOWING the establishment of an association between exposure to asbestos dust and the development of diffuse mesotheliomas of the pleura and peritoneum^{1,2} investigations into the relative risks of exposure to the various types of

Table 1 Average Measurements

	Age (years)	Body length (mm)	Lens mass (g)	Lens diameter (mm)
Group A	3+	113	0.016	3.04
Group B	7+	208	0.069	4.72

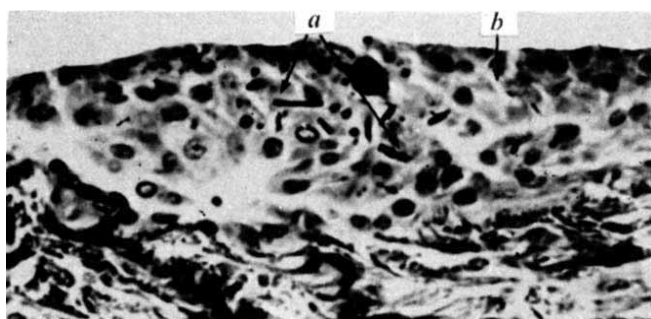


Fig. 1 Parietal pleura from 52 year old woman treated with asbestos (UICC Crocidolite) for 6 days. Note the proliferation of mesothelial layers of cells. ($\times$ c. 360.) a, Asbestos particles; b, mesothelial cells.

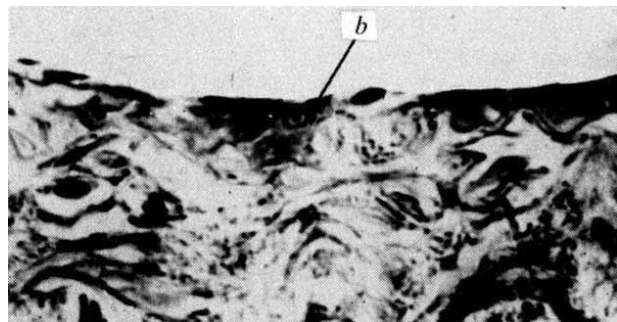


Fig. 2 Parietal pleura from same patient maintained in normal medium without asbestos. Note few mesothelial cells. ($\times$ c. 360.) Sections stained with azan.

asbestos fibre were made. Our studies were on an epidemiological³ and experimental⁴ basis. Experimental studies using both intrapleural inoculation and inhalation methods are time consuming as results cannot be expected in less than three years from the initiation of investigation, and a more flexible biological method was needed for these studies, because the major types of asbestos were found to have variations in structure and trace element associations. We therefore decided to investigate the possibilities of using organ culture techniques.

Human parietal pleurae obtained at open thoracotomy were dissected into 2 mm square pieces and were maintained in organ culture⁵ in a synthetic medium BGJ⁶ containing 15% foetal calf serum. Blue asbestos (UICC Crocidolite sample) was suspended in the medium at a concentration of 0.01%. A piece of pleura was maintained under identical conditions without the asbestos. The culture vessels were enclosed in a Fildes McIntosh jar in an atmosphere of 5% CO₂, 50% O₂ and 45% N₂. The medium was changed on alternate days and cultures were maintained for periods of up to 8 days.

Pleural explants in the presence of asbestos showed marked proliferation of the mesothelial cells (Fig. 1) when compared with controls in normal medium (Fig. 2). In some areas there was invasion of the underlying tissue by the cells and the cells had larger nuclei (7.0 μ m, s.e. 0.12) as compared with controls (4.5 μ m, s.e. 0.11). In addition there was an increased amount of collagen in the underlying tissue. This was evident in azan stained sections.

These experiments have demonstrated the ability of tissue in organ culture to respond to a fibre in a short period, compared with the long period necessary to produce similar effect *in vivo*. Organ culture systems should be useful in investigating the effect of various fibres, chemicals and perhaps carcinogens in a relatively short period.

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Received March 1, 1972.

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Radiochemicals: Variability of Quantity in 50 μ Ci Packages

WRITERS^{1,2} have warned of errors in the labelling of commercially purchased radioactive biochemicals with regard to content and purity. The next edition of the publication³ by the Committee on Specifications and Criteria for Biochemical Compounds, National Academy of Sciences—National Research Council, will contain a statement on definitions and problems involved in specifying the quality of radioactively labelled products. If a rigid testing procedure were followed by the user, errors in packaging would not cause difficulty, but it is likely that measurement of total activity, specific activity, nature of isotope and radiochemical purity, as recommended by Gordon², are an exception rather than the rule.

I wish to warn that the amount of isotope present in vials sold to contain 50 μ Ci may be in error and should be confirmed. Error limits for the amount present in the vials are not listed on the data sheets of any supplier. The only statement of variability in amount of material in a package that we have found appears in *User's Guide to Radiochemicals*⁴: "Amersham/Searle normally dispenses a few per cent extra (5 to 10%) o-tritium-labelled compounds to allow for losses due to adsorption of the compound often present in low (μ g concentration) on the walls of the container and for the natural decay of the radioactivity on storage. Carbon-14 compounds are usually close to the nominal content." Here are two examples of such problems, encountered in this laboratory.

Two vials of 2-¹⁴C-serotonin binosalate were found to contain approximately twice the stated amount (0.7 mg). The first vial contained 92 μ Ci of radioactivity; or 1.84 times the stated amount. The second vial contained 2.05 times the stated amount of radioactivity and 1.90 times the stated amount of solid by weight. The concentration of the solution measured at 275 nm using an ϵ value of 5,900⁵ was 1.55 times greater than the concentration calculated on the basis of the stated vial content.

Standards for purity and reference data for biogenic amines are not readily available. A subcommittee on Biogenic Amines and Related Compounds of the Committee on Specifications and Criteria for Biological Compounds has been appointed by the National Academy of Sciences, National Research Council, and will formulate tentative criteria for such compounds to provide reference data. The ϵ value for serotonin given by Bakri and Carlson⁶ at pH 7.9 is 4,300. Nishino *et al.*⁷ cite an un referenced value of 5,800. Obviously, better references are needed for checking these compounds.

Several years ago, 100 μ Ci of sodium pyruvate was received in two vials, marked A and B, 50 μ Ci and 1.7 mg each. The standard of radiochemical purity, checked by column chromatography⁸, was high. When analysed by the customary enzymatic assay for pyruvate, however, vial A contained 68% and vial B 151% of the listed amount. In answer to a query, the supplier stated that sodium pyruvate had been weighed on a semi-microbalance and that the weighing error of the tare and the final weight could be in error as much as ± 0.4 mg.

In some cases, vial contents are dispensed by the supplier as solutions, and in other cases by the weighing of solids. I recommend that reasonable limits of variability for both mass

and radioactivity be cited both on the label and in the supplier's catalogue for radioisotopes sold commercially.

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Confusion of Mirror Images by Pigeons and Interhemispheric Commissures

THE tendency in many species to confuse pairs of stimuli which are lateral mirror images of one another¹ may survive attempts to train animals to discriminate between mirror images. Even when such training has apparently been successful, the animal may actually distinguish the stimuli on the basis of cues other than left-right ones².

One explanation of mirror-image confusion is that when a memory trace is established in one hemisphere, a laterally reversed version is also transmitted through the interhemispheric commissures to the opposite hemisphere. Such a mechanism for mirror-image duplication of memory traces could have many natural advantages for an animal; it would enable an animal which encounters another in one profile subsequently to recognize the opposite profile. The suggestion follows naturally from the observation that many (but not all) of the commissural fibres connect homotopic mirror-image points in the two hemispheres^{3,4}.

Noble suggests that the prestriate areas of the visual cortex in monkeys maintain retinal topography, so that homotopic connexions between these areas, and possibly between the inferotemporal regions, would result in a left-right reversal of the retinal projection. But the commissures linking the visual cortices are probably not homotopic and are sparse⁵; in our view, Noble has overemphasized the importance of retinal topography. In addition he did not clearly distinguish interhemispheric perceptual transfer from interhemispheric memory transfer, which presumably occurs beyond the visual cortex¹.

If this explanation is valid, then mirror-image confusion should be abolished if the interhemispheric commissures are sectioned. Pavlov reported that a normal dog could not distinguish between a stimulus—being touched on one side of the body—and its mirror image. When the corpus callosum was sectioned, however, this discrimination was easily established⁶.

This result is not surprising, since somesthetic representation in each cerebral hemisphere is largely confined to the opposite side of the body. This makes it easy to understand why a transfer of tactual information from one hemisphere to the other might be accompanied by a switch in reference from one side of the body to the other. More compelling would be to

show that confusion of mirror-image visual patterns is abolished by commissural section, since the left-right sense of a visual pattern is not directly tied to the coordinates of the body surface, especially if the animal is free to view the pattern from any position.

We report data here which show that mirror-image confusion of visual patterns in pigeons is indeed abolished if the main interhemispheric commissures are sectioned. It has been demonstrated that normal pigeons trained to peck a key displaying an oblique line yield generalization gradients that are bimodal; there is one peak at the line they were trained to peck and another at its lateral mirror image⁷. Such pigeons, like Pavlov's dogs, are confusing mirror images. We have found that this confusion does not occur in split-brain pigeons.

Twelve homing pigeons maintained at 80% of their free-feeding weights were assigned to two groups designated "split-brain" and "normal". The split-brain birds were submitted to stereotaxic severance of the principal interhemispheric fibres.

There is conflicting evidence as to which commissures in the pigeon brain are essential for interhemispheric transfer of memory traces. A recent study by Meier⁸ has implicated the supraoptic decussation, but Gazzaniga⁹ has found that cutting the posterior commissure is sufficient to abolish transfer. Our decision was to cut the anterior, posterior, and tectal commissures, with a midline incision based on coordinates calculated from a stereotaxic atlas for the pigeon brain¹⁰. The operated birds were given several days to recover before training.

Table 1 Pecking Distribution for Test Pigeons

Split-brain group		Normal group	
Bird No.	No. of pecks	Bird No.	No. of pecks
2	922	8	800
3	382	9	676
4	275	10	212
5	423	11	578
6	617	12	444
		13	399

Training was carried out in a standard operant conditioning chamber containing a grain feeder and a translucent response key, onto which visual displays were projected from behind. The birds were first trained to feed from the grain feeder, and then to peck the key to operate the feeder. Reinforcement was 3 s access to grain in the feeder. The key displayed a white line sloping at 60° to horizontal, on a green background. The birds were given one session in which each peck on the key was reinforced, then five sessions of discrimination training in which two stimuli were alternately presented on the key every 30 s. During one of these stimuli (S+) pecks were reinforced after variable intervals averaging 1 min since the previous reinforcement (VI 1 min). No reinforcements were given during the other stimulus (S-). S+ was the 60° line on green background. S- was the green background only. By the fifth session of discrimination training >90% of pecking in all birds was during S+. On the day following the last day of discrimination training, the birds were given a test for generalization of pecking to six different slopes of the white line, ranging from 45° to 135° in steps of 15° (see Fig. 1 and Table 1). This test measured the extent to which the control by S+ over pecking was determined by the slope of the white line. S+ and the six new stimuli were presented in a mixed sequence, a stimulus change occurring every 30 s. Each stimulus was presented twelve times, and no reinforcements were given.

Following the experiment the split-brain birds were perfused with 10% formol saline and their brains removed and fixed in formalin for several days. Because of the extensive nature of the midline incision, severance of the commissures was

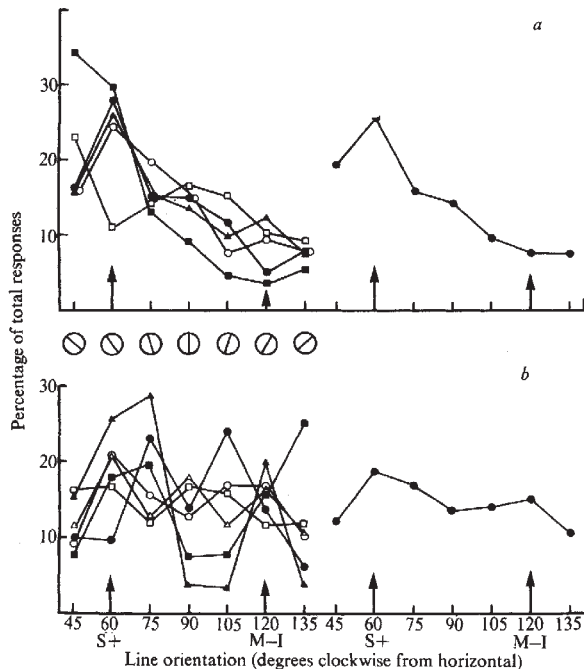


Fig. 1 Individual and group mean generalization gradients are shown. Individual gradients are based on percentage of total responding occurring in each stimulus. Mean gradients (right curves) are based on the means of these percentages. *a*, Split-brain group. *b*, Normal group. The raw means and standard deviations for the mean gradients, reading left to right, were: split-brain, 100.8/34.1, 133.4/75.9, 83.2/41, 74.8/38.4, 51.2/30.5, 40.4/43.9, 40.0/18.84; normal, 62.2/30.7, 95.5/44.01, 88.33/23.82, 69.5/46.7, 72.0/41.5, 78.5/28.7, 53.8/26.2.

verified by slightly parting the forebrain under an operating microscope and noting whether each of the three commissures was severed. In one bird the anterior commissure was not sectioned, and the results of that bird were excluded from the analysis.

Fig. 1 presents individual and mean generalization gradients for both groups of birds. The mean gradient for the normal birds shows a peak at both the training stimulus (60°) and its mirror image (120°). The mean gradient for the split-brain group is unimodal, peaking only at the training stimulus. Analysis of variance showed a significant interaction between groups and stimuli ($P < 0.05$). To probe the interaction further, trend analyses were carried out on the generalization gradients for each group separately¹¹. The normal group yielded only a significant quartic trend ($P < 0.05$), while the split-brain group yielded significant linear ($P < 0.001$) and cubic ($P < 0.05$) trends. This analysis confirms that the generalization gradients are bimodal for the normal group and unimodal for the split-brain group.

It is seen that the normal birds showed a tendency to respond to mirror-image sloping lines as equivalent, whereas the split-brain birds did not. This is consistent with the view that the interhemispheric commissures are critical to mirror-image confusion. This finding is of possible significance in understanding mirror-image confusions of reading and writing, commonly observed in children and in cases of dyslexia; it casts doubt on Orton's view that such confusions result from the direct formation of mirror-image traces in the two hemispheres¹².

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Imaginal Disks and the Abdominal Histoblasts of *Calliphora erythrocephala* (Diptera)

SINCE 1946 imaginal disks of *Drosophila* have been subjects for experimental morphogenesis³ because of their morphological discreteness, the long period they remain undifferentiated in the larva, and the ease of operation and transplantation. All the adult epidermal structures of cyclorhaphous Diptera are thought to derive from imaginal disks: head structures from labial, antennal, and eye disks; limbs, wings, and general thoracic epidermis from thoracic disks; genital apparatus from genital disks; and the abdominal epidermis from abdominal "imaginal disks"⁴ which, however, have been ignored because they differ from other imaginal disks. The adult cells of disks are set apart from the larval epidermis during embryonic development. Earlier workers have suggested a larval⁵ and prepupal⁶ origin of the abdominal "disks" in other species. Bautz⁷, in a study of the larval abdominal epidermis of *Calliphora*, shows that the "disks" are probably present from the time of hatching also. I have examined the abdominal epidermis of *Calliphora erythrocephala* throughout larval life, in whole mounts and in sectioned material by light and electron microscopy. Observations on the abdominal histoblasts show they should no longer be referred to as "imaginal disks", because the abdominal histoblasts differ not only in morphology but also in ultrastructural differentiation and developmental behaviour.

In each of the first seven hemisegments of the abdomen there are three groups of diploid cells, which even in freshly hatched larvae are discernible as more tightly packed columnar cells—eight in each dorso-lateral and ventro-lateral nest and four in a smaller postero-dorso-lateral nest⁷ (Fig. 1a). These histoblast islands become more distinct during larval life as the surrounding larval cells hypertrophy and their nuclei become highly polytene. The two larger groups of histoblasts in each hemisegment give rise in the adult to tergal and sternal structures⁸. Extirpation of the smaller group from third instar larvae has no effect on size or shape of these adult structures, but does interfere with the pattern of differentiated hairs. The fate of this anlage is not clear. Using the technique of clonal analysis, Garcia-Bellido and Merriam⁹ found that in *Drosophila* the minimum number of primitive anlage cells in the embryo which will later form a hemitergite is eight, and this remains constant throughout larval development. But in whole mounts of *Drosophila melanogaster* late third-instar larvae there are at least fourteen cells in the dorso-lateral group of histoblasts (M. J. P., unpublished observations).

This discrepancy could either be due to the clonal technique being uncertain with small cell numbers, or to a proportion of these dorso-lateral histoblasts forming structures other than the hemitergite. *Calliphora* differ from *Drosophila* in that during the growth of larvae the larger groups of histoblasts increase by cell division to about 300 cells, and to about 50 cells in the postero-dorso-lateral group, by the time of puparium formation.

Histological sections show that these histoblasts, unlike imaginal disks, do not separate from the epidermis by a peripodial stalk and are not enclosed by a peripodial membrane. Instead they lie within the larval epidermis (Fig. 1b). The basal ends of the cells are attached by hemidesmosomes to the basement membrane and the apical surface is in contact with the cuticle. Electron microscopy shows that the histoblasts of a mid third instar larva are actively secreting larval cuticle

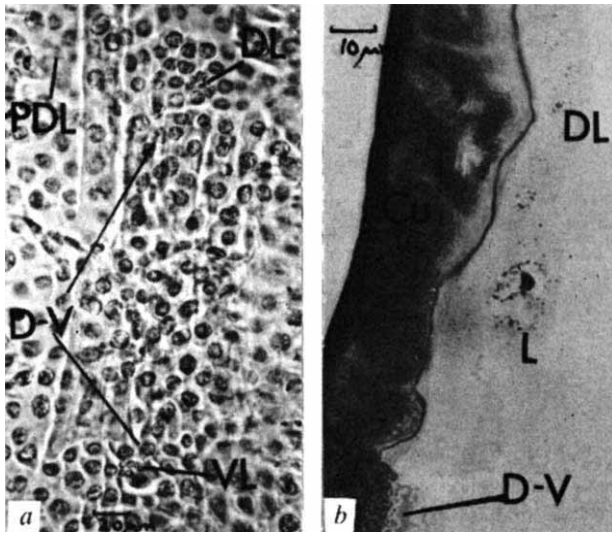


Fig. 1 a, Whole mount of left fifth abdominal hemisegment showing the dorso-lateral (DL), ventro-lateral (VL), and postero-dorso-lateral (PDL) histoblasts in relation to the lateral dorso-ventral muscles (D-V) in a first instar larva. b, Third instar abdominal epidermis in section, showing dorso-lateral histoblasts (DL) and hypertrophied larval cell (L) below the cuticle (Cu). A muscle insertion (D-V) is seen below.

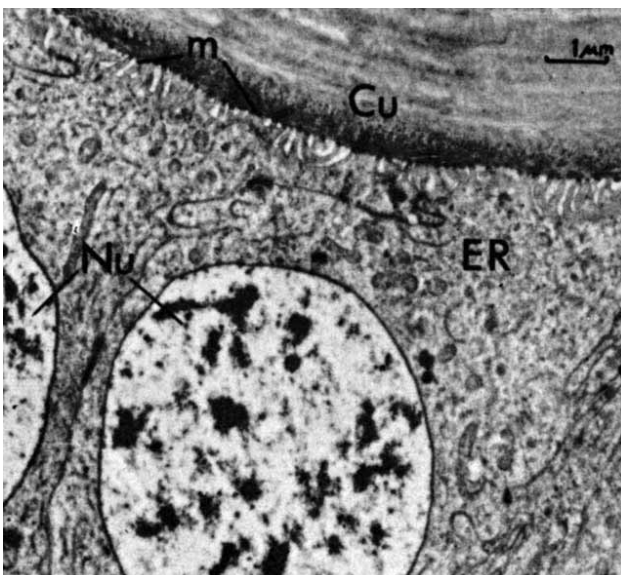


Fig. 2 Adult histoblasts of the fifth dorso-lateral group. Cuticular (Cu) filaments are seen laid down from the microvillae (m) of histoblasts whose nuclei (Nu) are shown. Rough endoplasmic reticulum (ER) is abundant. The section is taken from a three-day third instar larva.

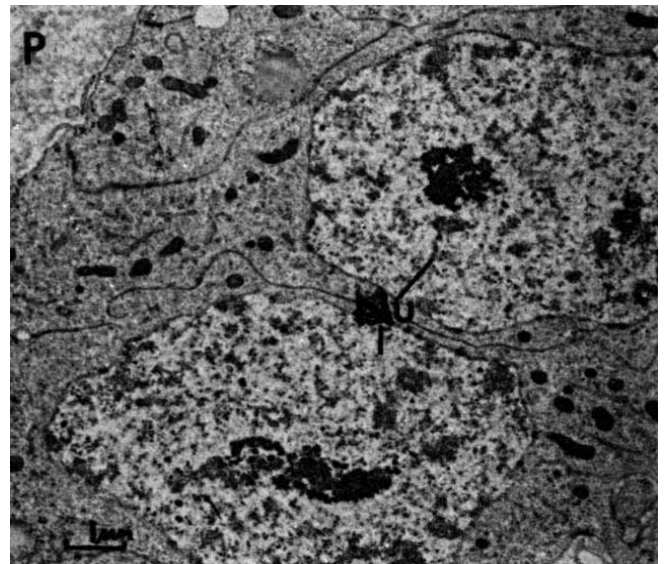


Fig. 3 Cells from the wing disk of a three-day larva. The nuclei of two cells are shown (Nu). There are many free ribosomes but little endoplasmic reticulum. P, Peripodial space.

indistinguishable from that formed at the surface of the polytene larval cells (Fig. 2). In both, cuticular histoblasts and polytene cell filaments pass into the lamellae from the tips of microvillae, and both possess an extensive rough endoplasmic reticulum and numerous mitochondria; a state of ultrastructural differentiation which differs markedly from the imaginal cells of wing disks (Fig. 3) or leg disks from animals of the same age and from imaginal disks in *Drosophila*¹⁰.

At the larval to "pupal" moult the thoracic and head disks are everted. The general thoracic epidermis at this stage is a layer of diploid adult cells which have spread from the peripodial stalks of the thoracic disks to replace larval cells, and the eighth abdominal segment has been remodelled by cells from the genital disk. The abdominal epidermis which secretes the fourth "pupal" instar cuticle still comprises larval cells and not until 36 h after puparium formation (at 25° C and a relative humidity of 90%) do cells from the adult anlagen, the abdominal histoblasts, invade the hypertrophied larval cells.

The developmental properties of these diploid histoblasts are less like the imaginal disks of higher Diptera and Hymenoptera, and more like the abdominal epidermal cells of hemimetabolous insects such as *Rhodnius* which not only secrete larval cuticle but also form the adult cuticular structures after metamorphosis¹¹. Similarly, in the abdomen of the holometabolous orders Coleoptera and Lepidoptera, the larval epidermal cells, which do not hypertrophy, retain the capacity, after a period of mitotic activity, to differentiate as adult cells^{12,13}. In these orders the larva does not grow rapidly, and because larval cells do not hypertrophy the necessity to retain a separate stock of diploid cells for redifferentiation does not arise.

The abdominal histoblasts do not show the properties of imaginal disks, i.e., the peculiar status as developmentally determined, undifferentiated systems destined to form adult structures incompatible with larval function. They are primitively diploid cells discretely organized in segmental anlagen among the hypertrophied larval cells which have become specialized for extreme larval growth.

This work was supported by an SRC grant. I thank Professor T. Weis-Fogh, Dr P. A. Lawrence, Dr B. L. Gupta and Dr C. M. Bate for discussion.

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Mouse Strain Identification

INBRED strains of mice have been widely used since 1909, and more than 200 distinct strains or sub-strains are now recognized². In practice, however, although each strain has a unique set of characteristics such as mean life-span, tumour incidence, behaviour, reproductive performance and blood group, different strains may be morphologically indistinguishable. Thus, for example, several of the widely used inbred albino strains cannot easily be told apart from the more common albino randomly bred strains. Unfortunately, although simplified biochemical methods for strain identification have been developed³, they are still too costly and laborious.

Table 1 Mice Used in the Study

Strain name	Type of strain *	Number	Age range (days)
A	I	29	51-103
A2G	I	30	59-164
C3H/He-mg	I	30	34-58
NMRI	PI	27	52-74
NZW	I	30	37-54
NZB	I	30	42-151
NZW × NZB F1	F1	30	46-65
LACG	O	53	31-86
B10.BR	CR	7	79-82
B10.A	CR	8	97-99
HR	O	8	73-81
C57BR	I	8	103-161
C57L	I	8	65-67
ICFW	I	7	64-76
LACA	O	7	52

* I=recognized inbred strain; F1=F1 hybrid; PI=part-inbred strain; O=outbred strain; CR=congenic resistant strain (inbred).

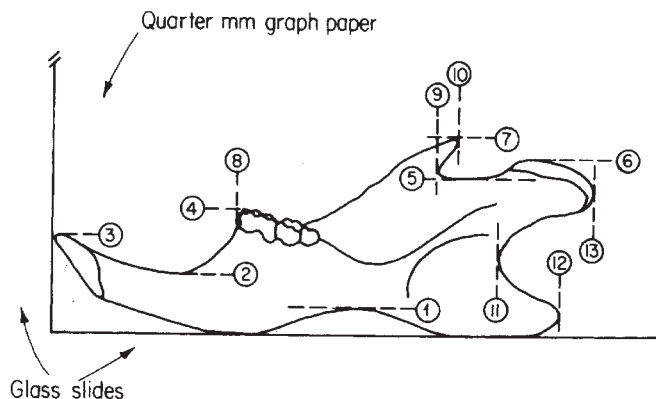


Fig. 1 Measurements taken on right mandible.

There are important skeletal differences between inbred strains⁴ and skeletal characters have been measured to study sub-line divergence in inbred mice⁶.

Techniques of multivariate morphometric analysis⁷ have been used by numerical taxonomists for a number of years, and have proved to be very powerful. I have attempted to use multivariate methods for the rapid identification of mouse strains.

Skeletons were prepared by papain digestion⁸. A method for measuring selected bones rapidly was then developed as follows: ordinary 1 mm graph paper was reduced photographically to a quarter size. Two double-thickness glass microscope slides were then glued to the print at right angles to form the ordinate and abscissa of a graph. Bones could be placed on this graph, touching the glass slides in a standard way, and the height and length of selected regions could be read off directly (to the nearest 0.125 mm) under a ×10 monocular microscope (Fig. 1). Thirteen measurements of the right mandible could be read off as quickly as they could be recorded by an assistant, and about 40 mandibles could be measured per hour. The technique is applicable to obtaining measurements of the mandible, scapula, atlas and pelvis and can also be used to measure the long-bones. Only the mandible was used in these studies.

The details of mouse strains used are given in Table 1 and elsewhere⁹. Most mice were over 6 weeks of age when killed, but a wide age range was used to reduce the effect of bone size. All animals were maintained under the same husbandry conditions⁹.

The statistical analysis was carried out using the standard computer program BMD 07M, "Stepwise discriminant analysis"¹⁰, which calculates the generalized distance function (squared Mahalanobis distance) that can give the probability that an individual is a member of each group, and classify all individuals into the group with the largest probability of group membership. (Similar programs are available elsewhere.) The program also calculates a pair of canonical variables which can be used to give a two-dimensional representation

Table 2 Classification Matrix

Strain	A	A2G	Number of cases classified into strain				C3H	NMRI	LACG	Unclassified
			NZW	NZW × NZB	NZB					
A	29	0	0	0	0	0	0	0	0	0
A2G	1*	29	0	0	0	0	0	0	0	0
NZW	0	0	30	0	0	0	0	0	0	0
NZW × NZB	0	0	0	30	0	0	0	0	0	0
NZB	0	0	0	0	30	0	0	0	0	0
C3H	0	0	0	0	0	30	0	0	0	0
NMRI	0	0	0	0	0	0	27	0	0	0
LACG	0	0	0	0	0	0	0	27	0	0
"Unknown"	0	2*	0	0	1*	0	0	25+1*	50	0

* Incorrectly classified mandibles. Only 5/312 were incorrectly classified.

of the scatter of individual members of a group and group means.

In the statistical analysis eight groups were defined with about thirty individuals/group (Table 2). Only one of 233 mandibles was misclassified in this analysis. Seventy-nine "unknown" mandibles were then tested: fifty-three were from other strains in Table 1, and twenty-six from strain LACG, one of the eight groups. The "unknowns" were recorded as belonging to a group if there was more than one chance in 100 of group membership. Two mandibles from strain HR were incorrectly classified as belonging to strain A2G, one mandible from C57BR was incorrectly classified as LACG, and one mandible from strain LACG was incorrectly classified as belonging to strain NZB. Thus, of the 312 mandibles studied only five were incorrectly classified. Using inbred, F1 hybrid, and non-inbred strains, 98% of the mandibles were correctly classified. Moreover, the technique could have been made considerably more precise if measurements on other bones had been included.

It may, therefore, be technically feasible to establish an international reference centre for strain identification, once samples of most of the known inbred strains have been measured. The technique also appears to be sufficiently precise to detect subline differences. Thus, further analysis showed that strains B10.A and B10.BR were genetically distinct, though closely related. The technique might therefore be used to study the development of substrains, and could possibly be used to re-define the conditions under which a new substrain is recognized. At present, new sublines are established simply by transferring stock from one laboratory to another¹ even though genetically there should be no important differences developing from a number of generations. The method can also be applied to closed random-bred stock, and probably to other species.

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Polar Auxin Transport in Leaves of Monocotyledons

ALMOST nothing is known about the establishment of cellular polarity underlying the polar auxin transport system of higher plants. Osborne¹ has suggested that the apical ends of cells derived from an apical meristem by sequential divisions are younger than the basal ends: their polarity and the basipetal transport of auxin are due to this age difference. Sachs² in his work on regenerating vascular strands has found that gradients of auxin may be responsible for establishing the cellular polarity and the subsequent transport of auxin in the direction of the initial gradient. Shoot tips and expanding dicot leaves contain relatively high levels of auxin. The basipetal polarity of auxin transport in petioles and stems is therefore associated with basipetal auxin gradients. In grass coleoptiles the greatest

amounts of auxin are found at the tip, where basipetal auxin transport is also associated with basipetal auxin gradients.

In monocot leaves which grow by a basal intercalary meristem, the pattern of cell division and of auxin distribution is more or less the reverse of that found in shoot tips. Sequential divisions of the basal meristem presumably make younger the basal ends of cells; and in growing monocot leaves the greatest amounts of auxin are found at the base^{3,4}. The polarity of auxin transport in monocot leaves is therefore of considerable interest.

Hertel and Leopold⁵ reported that in the primary leaf of *Zea mays*, auxin transport was basipetal. No other references to auxin transport in monocot leaves are available and I therefore tested the leaves of a number of species. In every case auxin transport was basipetal (Table 1).

Table 1 Auxin Transport by Sections of Young Leaves

Plant	C.p.m. in receiver blocks	
	Acropetal transport	Basipetal transport
Amaryllidaceae		
<i>Galanthus nivalis</i> L (8)	1	2,098
<i>Narcissus</i> sp (6)	0	4,616
Cyperaceae		
<i>Carex pendula</i> L (4)	6	553
Gramineae		
<i>Hordeum vulgare</i> L (6)	2	117
Iridaceae		
<i>Crocus purpureus</i> Weston (6)	0	142
<i>Iris</i> sp (4)	2	999
Liliaceae		
<i>Chlorophytum comosum</i> (Thunb.) Jacques (8)	2	51
<i>Cordylone australis</i> (Forst.) Hook. f. (4)	0	106
Orchidaceae		
<i>Cymbidium lowianum</i> Rchb. f. (4)	0	784
Palmae		
<i>Erythea armata</i> (Wats.) Wats. (6, leaflets)	1	52

Leaves were collected from plants growing outdoors and from tropical species in glasshouses. Sections (7 mm) were placed horizontally, supported by a strip of filter paper coated with petroleum jelly, on glass slides, and were supplied with agar (1% w/v) donor and receiver blocks. Donor blocks contained [¹⁴C] indol-3-yl acetic acid (52 mCi/mM, Amersham) at a concentration of 3.0 μM. During the transport period (3.5 h) the sections were kept in the dark in Petri dishes lined with moistened filter paper. Receiver blocks were then placed in scintillation vials with liquid scintillator⁶ (4 ml.) and counted for at least 10 min on a Packard TriCarb scintillation counter. Background counts (25–30 c.p.m.) were subtracted from the results. The numbers of leaf sections used are shown in parentheses.

Table 2 Auxin Transport in Primary Leaves

Region of leaf	C.p.m. above background			
	11-day-old plants		19-day-old plants	
	Acropetal transport	Basipetal transport	Acropetal transport	Basipetal transport
Tip	1	28	1	1
Middle	1	670	0	49
Base	0	525	0	106
Leaf-leaf sheath junction	1	495	1	107
Leaf sheath	2	346	0	117

7 mm sections were taken from different regions of primary leaves of *Avena sativa* plants grown in daylight at 22° C. Procedure as in Table 1. 10 sections were used for each sample. Transport time 3.5 h.

In leaves of young plants of *Avena sativa*, basipetal auxin transport took place across the meristematic region at the base of the leaf and also in the leaf sheath (Table 2), which grows by a basal meristem⁶. Plants germinated and grown in darkness yielded similar results. Less auxin transport was found near the leaf tip than in the younger, more basal parts of the leaf and younger leaves had a greater ability to transport auxin than older leaves (Table 2). A decline in the ability of cells to

transport auxin as they grow older has been observed in a number of other species and tissues⁷⁻¹⁰.

The hypothesis that polarity is determined by the difference in age between the apical and basal ends of cells becomes more complicated if it is to explain how both apical and basal meristems give rise to cells with a basipetal polarity. Its greatest weakness is in explaining the basipetal polarity of cambial derivatives¹¹ which arise not by transverse but by longitudinal divisions. The possibility that gradients of auxin determine the polarity of cells also seems at first sight unlikely to provide an explanation for the development of basipetal auxin transport in monocot leaves; but too little information is available about the anatomy of the basal meristems and the detailed pattern of auxin distribution in and around the meristematic region for any firm conclusions to be made.

I thank Professor F. G. Young for research facilities. I am a Royal Society Rosenheim research fellow.

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Received May 15, 1972.

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EBV Antibody in Sera of Non-human Primates

SEVERAL investigators have assayed sera of non-human primates for antibody to the Epstein-Barr virus (EBV). Gerber and Birch¹ reported the widespread distribution of complement-fixing antibody derived from the chimpanzee, baboon, rhesus and African green monkey. In a subsequent study, Gerber and Rosenblum² reported that many rhesus monkeys bled within 4 days of capture were also EBV seropositive, but Henle and Henle³ had previously failed to demonstrate EBV antibody using the indirect immunofluorescence method in sera from the chimpanzee, baboon and rhesus monkey. Two of 4 baboons, however, inoculated in our laboratory with 10⁸ EB3 cells were found by the Henles³ to have low levels of antibody 4-8 weeks after exposure. Landon and Malan⁴ have reported the presence of EBV antibody in rhesus and cynomolgus monkeys at birth. This antibody disappeared after 8-10 months, although sera from the mothers of these animals remained positive. Levy *et al.*⁵ were able to demonstrate antibody in 2 of 3 chimpanzees bled immediately after capture in the jungle. The number of animals used, species and geographic distribution, and finally the lack of history on the animals involved in all of these studies, plus the need for a suitable experimental host system, suggested that additional studies were worth pursuing.

Our primary interest in EBV antibody was two-fold: first, the presence of antibody in relation to phylogeny, and second, the presence of antibody in newly-captured animals. To answer these questions, sera were obtained from representative Old and New World monkeys and apes after varying periods of time in captivity, and from chimpanzees and baboons immediately after capture (Fig. 1). All sera were tested by the indirect immunofluorescent method described by Henle and Henle⁶, using goat anti-human IgG and P3HR-1 cells. Anti-

monkey (squirrel) gamma globulin conjugate (Dr V. Dunkel (NIH)) was also used, and gave essentially the same results. This material was used to exclude possible lack of cross reactivity when using anti-human preparations.

As shown in Fig. 1, only sera from the Old World monkeys and apes yielded positive reactions. All the sera from both gorillas and chimpanzees (with one chimpanzee exception) had antibody with titres ranging from 1:20 to greater than 1:160. Also, 6 chimpanzees bled at the time of capture were all seropositive (titres 1:10 to greater than 1:160), and antibody was also present in baboon and rhesus sera. It will be noted, however, that only half (approximately) of these sera were positive and the titres were somewhat lower especially among the baboons. Again, two sera from baboons obtained on capture were also positive. Both of the New World species tested, that is, marmosets and howlers, were completely devoid of EBV antibody at the 1:10 level.

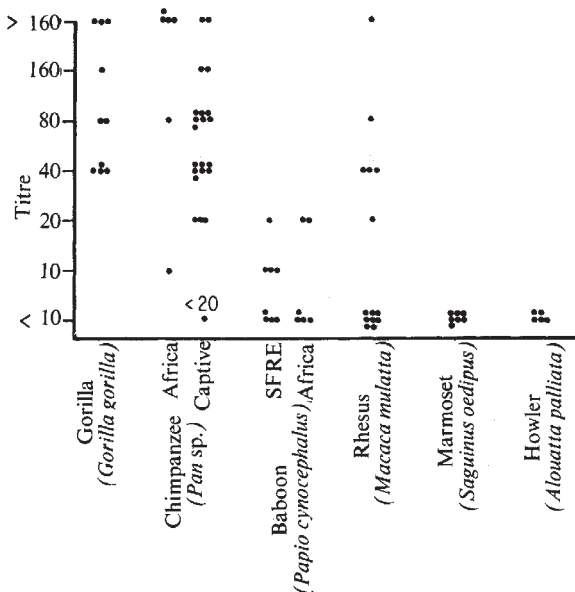


Fig. 1

Dunkel (personal communication) arrived at essentially the same results following testing of sera derived from the chimpanzee (*Pan sp.*), baboon (*Papio sp.*), rhesus (*Macaca mulatta*), cynomolgus (*M. fascicularis*), bonnet (*M. radiata*), African green (*Cercopithecus aethiops*), talapoin (*C. talapoin*), squirrel (*Saimiri sciureus*), and owl (*Aotus trivirgatus*) monkeys, marmoset (*Callithrix*) and the prosimian bushbaby (*Galago crassicaudatus*). The last four species were found to be negative.

The positive results obtained here are of interest, as they demonstrate a possible phylogenetic relationship to man as regards infection with EBV. Furthermore, the results also indicate a descending relationship as one passes from the apes down through the monkeys. Newly captured animals also have antibody, a finding that would suggest infection in nature with this or an antigenically similar virus, such as that causing infectious mononucleosis: this suggestion also has been put forward by Leby *et al.*⁵. While we are in agreement with this concept, our experiences in the wild⁷ make us cautious in stating that this is the only possibility. Both chimpanzees and baboons live close enough to man's environment to make it impossible to rule out human contact of some sort. These data would suggest, however, that a non-human primate "reservoir" for EBV may exist. Additionally the chimpanzee or baboon may serve as a model for the study of this virus.

This work was supported in part by grants from the US Public Health Service, the National Institutes of Health, and

the World Health Organization. We thank Bettye Tunmer and Wiebke Graham for technical assistance.

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Received January 17, 1972.

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Short-lasting, Competitive Neuromuscular Blocking Activity in a Series of Azobis-Arylimidazo-[1,2-a]-Pyridinium Dihalides

SUCCINYLCHOLINE is the most commonly used neuromuscular blocking drug for relaxing skeletal muscles during surgery or electroconvulsive therapy. Its main advantages are rapid onset and brief duration of action. It has, however, serious defects in that it often causes marked postoperative pain in skeletal muscles because its depolarizing action causes an asynchronous contraction in muscle bundles before paralysis occurs; there is no convenient remedy for controlling the prolonged apnoea which occurs in individuals deficient in serum pseudocholinesterase. Competitive neuromuscular blocking drugs such as (+)-tubocurarine or pancuronium do not cause muscle pain but are rather long-acting. Further disadvantages are that pancuronium has a slow onset of action and (+)-tubocurarine releases histamine and partially blocks autonomic ganglia at paralyzing doses. There is therefore still a need for a short-acting, competitive neuromuscular blocking agent with a rapid onset of action and which does not affect the cardiovascular system at therapeutic doses. Some of the azobis-arylimidazo-[1,2-a]-pyridinium derivatives described here appear to have these properties. Most of the compounds were made by Glover¹ and his colleagues at Tees-side Polytechnic; some were made in the research laboratories of Allen and Hanburys Ltd.

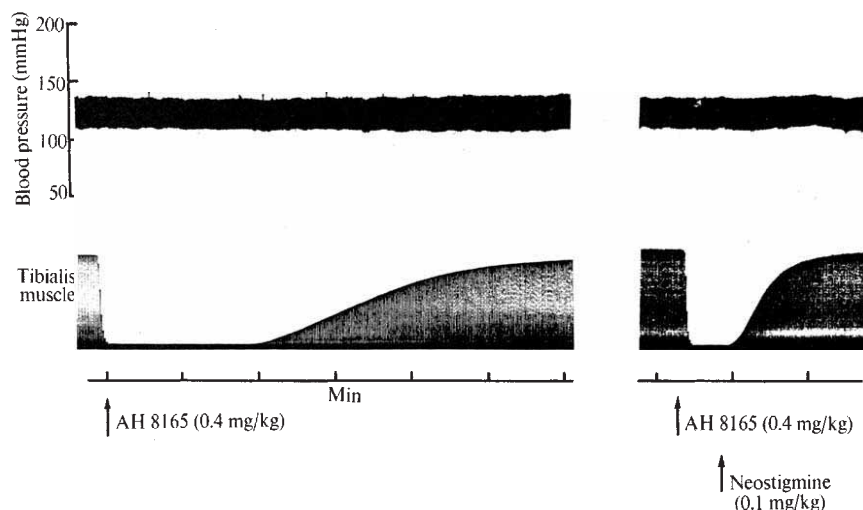
Table 1 Paralyzing Activities of some Azobis-Arylimidazo-[1,2-a]-Pyridinium Analogues, (+)-Tubocurarine, Succinylcholine, Gallamine and Pancuronium following Intravenous Injection in the Conscious Mouse

AH No.	R ₁	R ₂	Paralyzing activity	
			ED 50 (95% fid. limits) mg/kg i.v.	LD 50 (95% fid. limits) mg/kg i.v.
7394	H	H	> 10	> 10
8604	CH ₃	CH ₃	> 10	> 10
7336	Br	CH ₃	> 10	> 10
7060	H		0.35 (0.17-0.72)	0.70 (0.56-0.88)
7486	H		0.63 (0.48-0.81)	2.63 (2.03-3.38)
8117	Br		0.22 (0.16-0.29)	0.78 (0.56-1.09)
8165	CH ₃		0.16 (0.12-0.21)	0.31 (0.25-0.38)
8608		CH ₃	0.76 (0.66-0.87)	1.47 (1.27-1.71)
8607			0.48 (0.39-0.62)	1.68 (1.35-2.11)
(+)-Tubocurarine			0.05 (0.04-0.06)	0.10 (0.08-0.12)
Succinylcholine			0.18 (0.14-0.23)	0.42 (0.34-0.53)
Gallamine			0.86 (0.74-0.99)	1.7 (1.5-2.0)
Pancuronium			0.007 (0.006-0.01)	0.014 (0.012-0.016)

The compounds were prepared as dibromides or dichlorides. The reference drugs used were (+)-tubocurarine dichloride, succinylcholine dichloride, gallamine triethiodide and pancuronium dibromide. Here doses refer to the parent base.

A first estimate of paralyzing activity was made by intravenously injecting saline solutions of the compounds into male albino mice, weighing 18-22 g. Quantified results based on the rotating drum method of Collier, Hall and Fieller², and estimates of the acute toxicities of the compounds, are given in Table 1. All the active compounds caused flaccid paralysis which lasted for only 1-2 min without sign of muscle fasciculations. AH 8165, the most potent compound, was about as active as succinylcholine.

Fig. 1 Neuromuscular blocking and cardiovascular effects of AH 8165 in the chloralose-anaesthetized cat. Upper trace, arterial blood pressure; lower trace, maximal twitches of the tibialis anterior muscle elicited indirectly at 1 Hz. All drugs given intravenously.



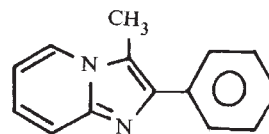
In 7–10 day old chicks AH 8165, 0.05–0.2 mg/kg intravenously, caused flaccid paralysis within 5 s followed by recovery in less than 1 min. More detailed studies with AH 8165 were carried out with anaesthetized cats, dogs and monkeys.

Cats were anaesthetized with chloralose, 80 mg/kg intravenously, following induction with 3% halothane in nitrous oxide and oxygen (3:1 v/v). The animals were maintained on artificial respiration throughout the experiments. Twitches of the tibialis anterior muscle were elicited at one second intervals by electrical stimulation of the peroneal branch of the sciatic nerve, by supramaximal rectangular pulses of 0.2 ms duration, the nerve being ligated central to the electrode. Muscle contractions were recorded on a 'Devices' M4 recorder and measured with a 'Statham' 32 oz strain gauge. Arterial blood pressure was measured from a cannula in a carotid artery and the pulse pressure used to trigger a 'Neilson' instantaneous ratemeter for a continuous record of heart rate. In some experiments, ECG (Lead II) was recorded using fine needle electrodes implanted subcutaneously; in others, contractions of the nictitating membrane to pre- and post-ganglionic stimulation were also recorded. Drugs were injected intravenously through a cannula in the jugular vein. AH 8165, 0.1–0.4 mg/kg, reduced maximal twitches of the tibialis anterior muscle in a dose dependent manner. For example, 0.2 and 0.4 mg/kg caused 34.7 ± 11.3 and $96.9 \pm 3.9\%$ neuromuscular blockade respectively. The blockade occurred within 10 s and recovery took only 1–4 min. In the same conditions (+)-tubocurarine, 0.1 mg/kg, caused $28.8 \pm 10.3\%$ blockade which lasted 8–10 min. Slow intravenous infusion of AH 8165, 0.06 mg/kg/min, produced near-complete neuromuscular blockade which could be easily varied by altering the rate of infusion. When the infusion was stopped after periods of 2–3 h, the muscle twitch tension returned to the pre-dosing levels in 5–7 min. The neuromuscular blockade induced by AH 8165 was never preceded by potentiation of the twitch response nor did the drug cause muscle fasciculations. AH 8165 and (+)-tubocurarine were alike in that the induced neuromuscular blockade was reversed by neostigmine, 0.1 mg/kg intravenously (Fig. 1), but they differed in that fully effective neuromuscular blocking doses of AH 8165 did not significantly affect the arterial blood pressure, heart rate or the ECG. Larger doses of AH 8165, 1–5 mg/kg, caused dose-dependent falls in blood pressure of 15–60 mm Hg which were sometimes accompanied by a slight increase in heart rate. The fall in blood pressure was not caused by a muscarinic action, histamine release or by activation of a vagal reflex pathway, because it was not prevented by pretreatment with atropine, mepyramine or by bilateral vagotomy. The hypotension was, however, broadly proportional to the degree of inhibition of the responses of the nictitating membrane to pre-ganglionic nerve stimulation; the responses to post-ganglionic nerve stimulation were unaffected by AH 8165 at these doses. It was therefore concluded that large doses of AH 8165 caused partial blockade of autonomic ganglia and thus lowered blood pressure.

In dogs and monkeys anaesthetized with pentobarbitone sodium, neuromuscular blockade was achieved with AH 8165, 0.05–0.2 mg/kg intravenously. As in the cat, effective blockade was rapid in onset, was short-lasting, could be rapidly reversed by neostigmine, and was not accompanied by significant effects of blood pressure, heart rate or ECG.

Both the urine and faeces were found to be important routes for the excretion of radioactivity after intravenous and/or subcutaneous injection of randomly tritiated AH 8165 in the rat and in the dog, but the excretion rate was complicated. Approximately 70% of the radioactivity was excreted within 24 h and the rest more slowly. Similar degrees of retention in the body have been reported after injected pancuronium³, stercuronium⁴ and probably (+)-tubocurarine⁴, and this may be a general property of bis-quaternary compounds which are capable of fixing to mucoproteins and other body sub-

stances which contain strongly acidic groups. Entero-hepatic circulation of metabolite 2 of AH 8165 (see below) may, however, account for much of the apparent persistence of the drug. *In vitro*, AH 8165 was rapidly reduced under anaerobic conditions by an NADPH-dependent azo-reductase present in liver microsomes. The end products were 3-methyl-2-phenylimidazo-[1,2-a]-pyridine (metabolite 1) and nitrogen.



Metabolite 1

After large doses of ³H AH 8165 *in vivo* unchanged drug, metabolite 1, and neutral, as yet unidentified, metabolite 2, were found in about equal amounts in the bile and urine from rats and dogs. After ordinary paralysing doses of ³H AH 8165, metabolite 2 was the major radioactive excretion product. Metabolic studies using ¹⁴C AH 8165 are in progress.

No significant abnormalities were found in dogs and rabbits repeatedly anaesthetized during a 4-week period with a variety of anaesthetics and paralysed for varying times with AH 8165. Studies in human beings are being arranged.

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Monoamine Changes in the Brain of Rats injected with L-5-Hydroxytryptophan

THE hypothesis of disturbed metabolism of indoleamine in endogenous depression is supported by the facts that 5-hydroxy-indoleacetic acid (5HIAA), a main metabolite of serotonin, is decreased in the cerebrospinal fluid^{1,2}, that serotonin concentrations in the hind brain obtained from depressed patients who have committed suicide are diminished³, and that 5-hydroxytryptophan (5HTP)⁴ and tryptophan⁵ can have a therapeutic effect. The efficacy of L-5HTP, the biological immediate serotonin precursor, in the symptomatic treatment of endogenous depression has been reported⁶, although no biochemical analyses of central monoamine changes following L-5HTP treatment have been made. We now give a description of L-5HTP-induced regional changes in central monoamine concentrations, and plasma corticosterone levels in relation to these changes.

Wistar male rats were given varying doses (10 mg, or 50 mg/kg of body weight) of a warmed L-5HTP solution (10 mg/ml.). Concentrations of serotonin, 5HIAA and norepinephrine in the five areas of the brain, striatum (STR), dorsal hippocampus (HIP), temporal part including amygdala (AMY), hypothalamus (HYPT) and midbrain (MB), were determined

60 min after intraperitoneal administration of L-5HTP. We used the methods of Maichel *et al.*⁷ to determine serotonin and norepinephrine, and Curzon *et al.*⁸ for 5HIAA. Plasma corticosterone was measured by the method of Mattingley⁹.

The monoamine concentrations of different brain areas after injection of L-5HTP are shown in Fig. 1. After the administration of L-5HTP in a dose of 10 mg/kg of body weight, serotonin content increased in HIP (1.8 times as much as in the control), STR (1.6) and HYPT (1.4). In MB and AMY, no significant differences were confirmed. 5HIAA content increased in all five areas, especially in HIP and STR, where it was 2.7 times; in other areas: 2.1. On the other hand, norepinephrine content decreased in HYPT and increased in MB.

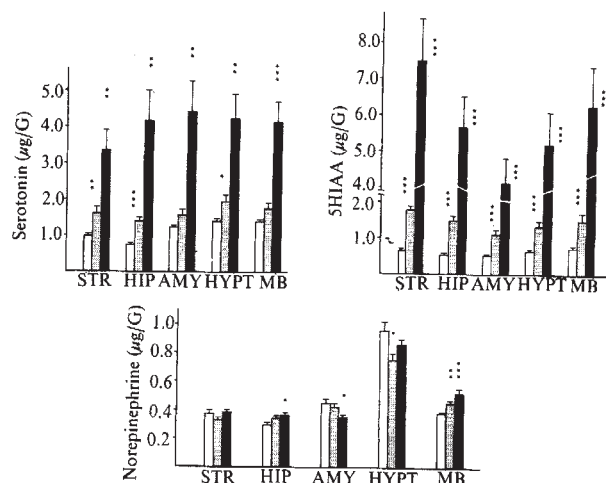


Fig. 1 Effect of L-5HTP on concentrations of biogenic amines in the brain of Wistar male rats. L-5HTP (10 mg, or 50 mg/kg of body weight) was injected intraperitoneally to rats. Control rats received saline. After 60 min, rats were decapitated and serotonin, 5HIAA and norepinephrine were analysed. Each group consists of 6 to 7 rats. Vertical bars indicate standard errors of the mean. STR, Striatum; HIP, dorsal hippocampus; AMY, temporal part including amygdala; HYPT, hypothalamus; MB, midbrain. White columns, control; stippled columns, L-5HTP 10 mg/kg; black columns, L-5HTP 50 mg/kg. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.

At a dose rate of 50 mg/kg of body weight, serotonin content rose in all five areas and the highest increase, compared with its control, was seen in HIP (5.5 times). 5HIAA content also increased markedly in general, especially in STR (11.5) and HIP (10.5). Norepinephrine content increased in MB and HIP, but decreased in AMY. Only at this dosage did plasma corticosterone show a statistically significant rise over the control level (Table 1).

Table 1 Effect of L-5HTP on Plasma Corticosterone

	Plasma corticosterone $\mu\text{g/dl} \pm \text{s.e.}$
Control	20.0 ± 3.3
L-5HTP 10 mg/kg	27.6 ± 4.1
50 mg/kg	$50.2 \pm 4.2^*$

* Differs from control rats at $P < 0.001$. Each group consists of 6 to 7 rats.

It has already been reported that the serotonin concentrations of the whole brain and different brain areas in several animals increased after administration of DL-5HTP¹⁰⁻¹² and the increase rate was most marked in the hippocampus of rabbits¹². Different structures in the brain have been shown to possess varying degrees of activity of 5HTP-decarboxylase¹³. The hippocampus has *in vitro* a greater enzymatic activity than

other cortical areas in dogs. According to Kuntzman *et al.*¹⁴, the caudate nucleus in cats has the highest activity of this enzyme. Thus the high rate of serotonin concentrations observed in the hippocampus and caudate nucleus in rats is considered to be dependent, in part, on the distribution of this enzyme.

Roos¹⁵ reported that the distribution of 5HIAA in the hemispheres and brainstem of rabbits was similar to that of serotonin after the injection of 5HTP. We found that the rates of increase of 5HIAA concentrations in the striatum and hippocampus were higher than those in other areas, as observed in serotonin concentrations.

L-DOPA, widely used for the treatment of Parkinson's disease, is reported to reduce central serotonin stores^{16,17}. Conversely, in patients treated with L-5HTP, changes of catecholamine stores in some areas of the brain may occur, in addition to the elevation of brain indoleamine concentrations, as observed in the different regional changes of noradrenaline concentrations after the administration of L-5HTP in varying doses. These findings may have important clinical implications in the treatment of endogenous depression with L-5HTP.

L- and DL-5HTP has been reported as ineffective in stimulating adrenocortical function in men^{18,19}. Our studies show that a large dose of L-5HTP is capable of increasing plasma corticosterone level, and this finding may be related to the regional monoamine changes in the brain, especially to the increased serotonin level in the hippocampus. DL-5HTP does, however, induce several peripheral actions similar to those produced by serotonin¹¹.

The animals injected with the larger dose showed a decreased voluntary movement combined with general muscle tremors. Therefore, this rise of plasma corticosterone level may be regarded as a result of non-specific stress induced by this amine.

Further investigation is required before the role of these changes of central monoamines in the regulation of the hypothalamo-hypophysial function can be established.

We thank Professor N. Suwa for support.

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Received April 11, 1972.

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BOOK REVIEWS

Water as a Resource

Aquatic Chemistry. By Werner Stumm and James J. Morgan. Pp. xv+583. (Wiley Interscience: New York, 1970.) \$24.95.

Water and Water Pollution Handbook. Edited by Leonard L. Ciaccio. Vol. 1: Pp. xvi+449. Vol. 2: Pp. ix+349. (Marcel Dekker: New York, 1971.) \$27.50 each vol.

Principles of Water Quality Control. By T. H. Y. Tebbutt. Pp. ix+179. (Pergamon Press: Oxford, 1971.) £2 cloth; £1.50 paper.

Biology and Water Pollution Control. By Charles E. Warren. Pp. xvi+434. (Saunders: Philadelphia, 1971.) \$11.00.

AMID the welter of books on "pollution", "ecology" and "the environment" intended for the general public, the decision-maker and other non-specialists, it is as well to recall that many worthy books on the scientific aspects of these subjects have been made available over the years to those who are professionally involved in water quality control. Many of the earlier textbooks had their origin in the pollution control measures which necessarily had to be taken in this country in the latter part of the last century; but more recently, with the growth of many schools of public health engineering in the US, an increasing proportion of such books has been of transatlantic origin. In spite of differences in emphasis, in climatic conditions, and in legal systems, pollution problems of different countries have much in common, and books on the subject have an increasingly international appeal. This is well shown by the present batch of recent publications.

Stumm and Morgan have written a book uncompromisingly for the water chemist; although it is modestly subtitled "An Introduction Explaining Chemical Equilibria in Natural Waters" one can hardly regard this detailed work as a mere introduction. It is a teaching book intended for advanced undergraduates who are expected to have "some" knowledge of chemistry. Numerical examples are used freely and each chapter concludes with problems and suggestions for further reading. It is the authors' expressed intention to deal with aquatic systems of all kinds ranging from natural waters to water treatment processes. Among the subjects receiving particular attention are

equilibria between solid phases and aqueous solutions, coordination chemistry of metal ions, and redox systems in natural waters. The book is detailed, comprehensive and economically written: it is clearly destined to become one of the standard works of reference.

The "Handbook" edited by Ciaccio is designed as a four-volume work, of which two volumes are now available and the others are due to appear during the current year; it is intended for a wide readership—those entering the field, those specializing in some aspect of it, and the multi-disciplinary scientist. The editor's aim has been to stress the need for adequate analytical techniques "to define what has happened, is happening, will happen, and where it may take us", the term analytical being used in the sense of a multi-disciplinary approach including chemical, microbiological, mathematical and other methods.

Part I of the work, forming volume 1, is concerned with the discussion of environmental systems, nine authors dealing with such subjects as water resources, estuaries, waste water treatment and the mathematical modelling of natural water systems. Part II is concerned with analytical techniques and will be published in volumes 2-4; volume 2 contains monographs on, *inter alia*, sampling, concentration and separation, bacterial and viral analysis, and toxicity bioassays.

The work illustrates the strengths and the weaknesses of the handbook approach: by commissioning a separate author for each chapter the editor can attempt to enlist the services of an expert on each specific topic. On the other hand, in spite of careful editing, differences in style and coverage are likely to become apparent. These monographs are well presented and documented, though the index promised in volume 4 is sorely needed. There is a tendency in some chapters (notably that on estuaries) to cover only the US literature.

Tebbutt's little book, the only indigenous member of the group, is primarily intended for undergraduates and other students. It is relatively undemanding in the level of scientific knowledge required for its readers and within its limited compass attempts to deal with the fundamentals of water pollution, the treatment of waste waters, disinfection, water reclamation and sludge disposal. Inevitably, therefore,

the treatment of each topic is very brief, though the book has some value as a rapid introduction to the subject. Some of the chemistry, particularly in the chapter on disinfection, is very shaky, and the author does not always make a clear distinction between processes which are theoretically feasible and those employed in large-scale practice. Problems and an index are provided.

The book written by Warren in collaboration with Peter Doudoroff is intended for a wide readership, including engineers, politicians and the interested public, and may well have some success in this respect since it combines sound scientific material with a critical approach, expressed lucidly and with imagination. Subjects dealt with include water quality standards, adaptation, and the ecology of individuals and populations, concluding with a consideration of the roles of water pollution biologists. Indeed, it is to this latter group that the book may make its greatest appeal, not least because it contains a great deal of hitherto unpublished material from the author's school at Oregon. This is a readable and thought-provoking book, and for those who do not wish to read it from cover to cover a good index is provided. Its title promises a rather more comprehensive coverage than is actually achieved, but nevertheless this is a very welcome addition to the literature on water pollution.

G. E. EDEN

Models of Behaviour

Feedback Mechanisms in Animal Behaviour. By D. J. McFarland. Pp. 279. (Academic: New York and London, December 1971.) £3.80; \$11.50.

In a literature overburdened with competent mediocrity, there are a few authors who can be relied upon to make an original contribution with every paper. In the field of animal behaviour Dr McFarland is one of the creative intelligences. He has shown that mechanisms of behaviour can be successfully inferred from external behaviour, using the gentle and subtle arts of the control engineer. He has written an important book, which all serious animal behaviourists should try to understand. The non-mathematical may find it difficult—I certainly did—although much of it can be understood on an intuitive level.

Perhaps it should have been read by a stupid critic before rather than after publication.

Undisciplined model-building is a menace, and can give the whole art a bad name. McFarland's theoretical creativity is matched by his experimental ingenuity, and it is the elegance of his own results which especially gives confidence in the value of his methods. Curiously, I found the examples drawn from other people's work particularly difficult to read.

The book shows signs of having been put together in a hurry. For example the preface and chapter 3 were presumably not really intended to start with the same paragraph, and the last sentence of the chapter 3 version of the paragraph does not make sense. The drawing on the cover is not well chosen.

One feels that the title may have been outgrown during the writing of the book. There is more here than feedback mechanisms. Chapter 1 introduces the idea of mathematical analogy and the "generalized variables", "effort", "momentum" and so on which are later applied to behaviour. It also equips the reader with a basic dictionary and grammar of control theory. Chapter 2 introduces feedback concepts. Chapters 3, 4 and 6 show how engineering techniques for analysing transfer functions of systems can be applied to behaviour. Chapter 5 discusses "errors" and describes some of the statistical techniques used by ethologists. The long final chapter is largely a stimulating review of the motivational theories of both psychologists and ethologists from the engineering viewpoint. The re-evaluation of the maligned "energy models" is provocative. The book ends with some exciting new ideas on the application of optimization theory to motivation, and one is left with the feeling that a new edition cannot be far away, such is the pace of the author's own inventive output.

RICHARD DAWKINS

Endocrine Elements

An ABC of Endocrinology. By K. J. Catt. (Reprinted from the *Lancet*, April 11 to August 15, 1970. With two additional chapters.) Pp. 154. (The *Lancet*: London, November 1971.) £1.

THIS new up-to-date summary of endocrinology can be strongly recommended as suitable for students in both medical and science faculties. The information provides an excellent synopsis of modern endocrinology but of necessity is too brief to serve as a full textbook. This was not, however, its intention. In spite of the brevity of the treatment, the information is well inte-

grated, covering both clinical and non-clinical examples, and the book has already proved invaluable to me for both senior science undergraduate students and postgraduate medically qualified staff following membership qualification courses. The diagrams are plentiful and well organized, and it is a pleasure to see such a useful text both well printed and reproduced and available in paperback form at a modest price. I thoroughly recommend it.

KENNETH A. MUNDAY

Knotted Molecules

Catenanes, Rotaxanes, and Knots. By Gottfried Schill. Translated by J. Boeckmann. (Organic Chemistry: a Series of Monographs.) Pp. x+192. (Academic: New York and London, February 1971.) \$11; £5.15.

THE pioneering studies by Ruzicka and Prelog of the chemistry of large and medium-sized cyclic molecules were a necessary prelude to the examination of the synthesis of chain-like molecules in which the components are not joined together by chemical bonds, but are just held together mechanically. Molecules of this type are known as catenanes and, although they were first recognized as a synthetic challenge by Willstätter during 1900-1912, it is only during the past decade that their synthesis has been achieved.

Professor Schill and his co-workers of the University of Freiburg have made outstanding contributions in the field of catenane and rotaxane chemistry, and this book is essentially a personal record of their achievements. A large part of the synthetic work described in this monograph has not been published previously, but presumably the experimental details will be made available in full papers shortly. This plea is made because some of the experimental techniques which have been used are novel. This account will be complementary to the full papers because after reading this monograph one can appreciate the philosophy and tactics which have been used in the design and exploration of synthetic routes which ultimately led to the successful synthesis of catenanes and rotaxanes.

The next objective in this field is the synthesis of molecular knots. This has not yet been achieved, but the last chapter contains an interesting appreciation of the concepts to be considered in the design of projected synthetic routes to knots, doubly wound catenanes, and rotaxanes.

This subject demands clearly drawn diagrams and formulae, and their presentation in this book is excellent.

W. D. OLLIS

Making Molecules

Biosynthesis of Macromolecules. By Vernon M. Ingram. Pp. xiii+301. (W. A. Benjamin: Menlo Park, California, 1971.) \$10.95 cloth; \$5.95 paperback.

IN negotiating the swiftly moving currents of molecular biology the author of a modern text on this subject must steer a skilful course between the Scylla of writing a volume that is already obsolete on publication and the Charybdis of including all the hottest—and often inaccurate—unconfirmed results. In his updated edition of *Biosynthesis of Macromolecules* Dr Ingram has admirably avoided these twin traps and has produced a lucid account that should prove to be a solid and lasting reference book for both students and practising scientists alike.

After charting his course Dr Ingram considers in turn the intricacies of DNA synthesis, RNA synthesis, protein synthesis and finally the genetic control of primary protein structure. Throughout the emphasis is on experimental fact. In every field the key experiments are described in detail and interpreted according to the strict canon of logic. The reader is left in no doubt as to what the experiment in question does—or does not—prove. Where doubts and gaps in our knowledge remain or interpretations are ambiguous, this is clearly stated. This approach allows a detached and refreshing analysis of contemporary concepts in molecular biology and compares favourably with the instant compilations of scientific papers which are now so much in vogue. Not that Dr Ingram neglects the original papers: on the contrary the reference list is comprehensive enough for the research scientist to use the book as a source of important references outside his or her own immediate field. It is a welcome sign of the importance assigned to experimental practice that the book includes a chapter describing and evaluating methods of sequencing biological macromolecules.

Only very occasionally does the normally impeccable oarsmanship of Dr Ingram show signs of catching a crab. Where it does, it is on such trivial snags as whether or not the -CCA end of tRNA molecules is transcribed by the DNA dependent RNA polymerase. Where the rare errors of fact occur, they can be attributed not to the author but to the original scientific paper to which he refers.

All in all, this is an advanced textbook about molecular biology as it is practised, written by a practising molecular biologist for those who are already practising and for those who

wish to do so. As such this book can be recommended without qualification.

ANDREW TRAVERS

Elihu Thomson

Selections from the Scientific Correspondence of Elihu Thomson. Edited by Harold J. Abrahams and Marion B. Savin. Pp. xiv+569. (MIT Press: Cambridge, Massachusetts, and London, 1971.) \$17.50.

THIS book contains a remarkable collection of letters written and received by an American engineer and scientist of great distinction. Elihu Thomson died in 1937 at the age of 84, and during his lifetime made a major contribution to the science and practical application of electricity as well as to a lesser though significant effect in other fields as evidenced in his patents numbering over 700. During his early years he formed a close friendship with a teaching colleague, Edward Houston, an association which later developed into the world-famous electrical concern, the Thomson-Houston Company.

The greater part of the correspondence is of course devoted to scientific work, and rarely has a man based his whole life philosophy more on the Latin motto "*Rerum cognoscere causas*". On January 29, 1930, in a dramatic reply to a former student requesting information on the history of his discovery of the Maxwellian wave, he recounts in a letter of a length equivalent to about three columns of *The Times* how, at the age of 22, "for the first time in the world's history that one circuit was tuned to correspond in its constants with another by making the minute changes in its capacity by hanging bits of foil on a wire". He admits that Houston was with him in these experiments, but says, "I do say the ideas were mine." In any case when he raced to the top of the building to find the waves had travelled a hundred feet Houston was not there, "he weighed 186 pounds stripped". The outcome of all these exciting events was of course that Elihu Thomson in 1875 anticipated Marconi by three years in the production of electro-magnetic waves.

An impressive side of Elihu Thomson's character emerges from the wide variety of subjects in which he took a keen interest and carried out pioneer work. From the correspondence between 1899 and 1916 with Hewitt, a distinguished architect and gifted amateur astronomer, and a longer one of 46 letters between 1902 and 1935 with Hale, the one-time Director of the Mount Wilson observatory, it is evident that the production of special glass and of large mirrors of fused quartz occupied much of his time. The step-by-

step progress of the work at Lynn makes a sensational story but one which seems, however, to have come to an end with the courageous prediction of a mirror 200 inches in diameter, 30 inches thick and weighing between 25 and 30 tons!

In the longest of the series covered by this book, sixty letters in all, Elihu Thomson and his favourite pen-friend Crompton had much in common. First of all they shared a keen sense of humour: Elihu's specific treatment against influenza was "... to get into contact with it as often as possible ... an attack limits itself by immunity ... if another attack can be obtained at the earliest possible time it will be but a light one". Crompton's ideas on health were rather different: "My invention is to apply radiant heat on the front side of the leg up to the knee. I find that keeping the head cool one does far better brain work." And in his last letter on December 11, 1935, he finds in his 91st year that, though his memory is to some extent reduced, his mental faculties are not much impaired.

One of the striking aspects of this collection of letters is the number of instances which anticipate future significant developments in science and invention. Throughout the correspondence much light is thrown on the personal character of Elihu Thomson which appears in so many of his letters and can be confirmed by me when I had the pleasure of meeting him in his laboratories at Lynn, Massachusetts, in 1926. He was a lovable man impressing all by his modesty and by the deep careful thought which he gave to any complex matter before declaring an opinion. This book is a wonderful tribute to a great man.

PERCY DUNSHEATH

Schneirla's Ants

Army Ants: A Study in Social Organization. By T. C. Schneirla. Edited by H. R. Topoff. Pp. xx+349+8 plates. (W. H. Freeman: San Francisco, 1971.) \$12.

THE nomadic Dorylines have become widely known, from works of fiction, as invading armies of ants destroying everything in their path. In fact each colony of African driver ants (*Anomma wilverthi*) has been estimated to consist of more than 10,000,000 ants, but their destructive powers have frequently been exaggerated. *Eciton* colonies, normally numbering at least 1,000,000 ants, fail to take half of the potential victims in the area raided and active vertebrates usually escape.

At the time of his death in 1968 Schneirla had been studying these phenomenal insects for nearly 40 years and eleven chapters of this book had

already been written by him, leaving three to be completed by Topoff. As expected from the author's justly famous earlier publications the central theme of the book is the control of cyclic periods of activity especially in genus *Eciton*. Each colony normally has one queen which lays eggs in batches separated by a month without laying and the colony is not nomadic while the queen's gaster is swollen with eggs. While each batch of larvae is growing the colony moves each night, but forms temporary "bivouac" nest sites each day and the queen is fully mobile with a reduced gaster. When the larvae cease growing and spin cocoons the colony ceases to be nomadic, the food intake is switched to the queen and the cycle starts again. Schneirla concludes that the cycle is brood determined and each nomadic phase is initiated by the hatching of a new batch of callow workers.

As well as the detailed account of *Eciton* cycles there is a chapter on colony division and much interesting comparison with other Dorylines, especially the much simpler *Aenictus*.

Unfortunately the book has faults which reduce its readability. Repetition of information and ideas soon becomes irritating and it was also unnecessary to repeat the colour illustrations in black-and-white or to repeat quotations such as that from Belt appearing on pages 7 and 43. Although the style is claimed to be semipopular it resembles that of scientific papers and in the later chapters there is a fashionable but deplorable tendency to state the obvious in complicated language. The space could better have been used to compare Dorylines with other ants as it is possible for readers to assume that some of the features described are special to Dorylines when they are actually more widespread.

One of the main impressions left on the reader is the high proportion of untested hypotheses presented and it would be appropriate if this book stimulated further field experiments on these fascinating ants—an approach particularly advocated by Schneirla.

A. J. PONTIN

Dating Samples

Dating Techniques for the Archeologist. Edited by Henry N. Micoale and Elizabeth K. Ralph. Pp. xi+227. (MIT: Cambridge, Massachusetts, and London, November 1971.) \$12.50.

ACCORDING to the blurb, this book has been written on the premise that many archaeologists need to be better informed on modern methods of dating, and it attempts to remedy this situation by discussing the technicalities of the various methods so that the archaeologist will

gain enough information to enable him to collect and prepare his samples properly.

The space allocated to the various techniques varies widely, and by no means in direct proportion to their relative importance to archaeology; archaeomagnetic dating (60 pp) is the longest chapter, and fission track (5 pp) the shortest, with carbon-14 (48 pp) running third. Undoubtedly the differences in lengths of chapters partly reflect the extent to which the various authors have considered it necessary to discuss the technical details of their subjects, and one finds here some examples of the way in which the specialist's enthusiasm for his subject can lead him into dealing with some aspects of it in more than necessary detail. For example, the chapter on carbon-14 dating achieves a good balance between brevity and detail in describing the basis of the method, samples and sampling, interpretation of results, and radiocarbon age discrepancies, but deals with laboratory techniques and particularly that of proportional gas counting in what may seem to be unnecessary detail to the archaeologist. Technical detail also tends to obscure the section on archaeomagnetic dating from the layman's point of view, although this is admittedly a very complex subject and one appreciates the author's difficulties. In contrast one can agree that the wealth of detail in the section on obsidian dating is fully justified because this is a technique which by virtue of its low cost and relative simplicity should be within the technical competence of a small archaeological unit to set up and operate, and the chapter provides enough practical detail to make this possible.

Finally, in commenting on a book aimed at enabling the archaeologist to collect and prepare his samples properly, one must make the point that some chapters could have devoted more space to the practical aspects of sampling, or alternatively specifically warned the archaeologist against attempting it in those cases where, as in archaeomagnetic dating, the technique is highly specialized.

HAROLD BARKER

Sociology of Science

The Social Process of Innovation. By M. J. Mulkay. Pp. 64. (Macmillan: London and Basingstoke, April 1972.) 60p.

IN this well-constructed essay, the author addresses himself primarily to the question: what sorts of scientists are the most likely to produce innovations? For this, he distinguishes between "migration" to nascent specialities and "radical innovation" that challenges existing paradigms. His description of

the social process of control of research makes effective use of the concepts of "cognitive and technical norms" and also the (unstable) "research network" which enforces these on workers in each problem area. Partly by a relatively formal argument using these concepts, and partly by commonsense argument at crucial points, he arrives at a tentative conclusion: high-status men will tend to migrate, and young men to innovate radically.

Always maintaining an admirable clarity concerning his assumptions and methods, the author articulates a model of *Homo scientificus*. He is a direct descendant of *Homo economicus*, an entrepreneur operating on a market whose currency is prestige, and as always concerned only to get the highest price for the product of his labours. The author explicitly defends this model: "It is sometimes argued that the scientist's main reward is neither money nor recognition but rather the pleasure derived from completing a successful piece of research. However, this argument is inconsistent with the regular occurrence of priority disputes and the prevalence of anxiety over anticipation."

In accordance with this model, the psychology of the young scientist prone to radical innovation is simply that of a gambler with little to lose and much to gain. An important part of his motivation is his ignorance that he is playing with a stacked deck: "Furthermore, it seems probable that young scientists will believe more firmly than their elders in the myth of scientific impartiality. If this is so, they will be less likely to conceive that revolutionary contributions might be rejected." The author's chosen case study of innovation is the work of Pasteur on fermentation; and so he apparently believes that the great scientists of the past, who endured hardships of many sorts during their struggles for acceptance of their ideas, were all silly little opportunists who struck it lucky.

It is to be regretted that the author's useful bibliography did not include N. Storer, *The Social System of Science* (1966), which in spite of its naiveté is valuable as the prime source of the "exchange" model of scientific activity: Two more recent studies indicate problems that could well be worth considering in connexion with such models of *Homo scientificus*. I would be interested to see if the maintenance of quality-control in science can be explained on such Hobbesian assumptions. And to this model of scientific activity, even more than to Kuhn's, there can be applied the Feyerabend paradox: is there anything in this analysis that does not apply equally well to gangsterism; and if not, why not?

J. R. RAVETZ

Simple Liquids

Physical Chemistry: An Advanced Treatise. Vol. 8. *Liquid State.* Vol. 8A, Pp. xix+1-412+14; Vol. 8B, Pp. xix+413-892. Edited by D. Henderson. (Academic: New York and London, December 1971.) Vol. 8A: \$23; £10.75. Vol. 8B: \$25; £11.65.

THIS treatise on the theory of molecularly simple liquids has fifteen authors, most of whom have concentrated on the developments of the last five years. Some of their material is in other books but there is here much which can be found elsewhere only in research and review papers.

Scott opens with a summary of our knowledge of intermolecular potentials; Chen and Baxter in separate but overlapping chapters discuss the determination of the structure of a liquid and its representation in terms of the distribution functions; Ree describes the computer simulation of model systems; Jhon and Eyring return to the latter's "significant structures"; and Henderson and Barker bring part A to a close with a welcome account of the perturbation theories with which they have enriched the armoury of equilibrium statistical mechanics. In part B, Henderson and Leonard apply these and other theories to liquid mixtures; ter Haar disposes of liquid helium in twenty-five pages; Berne gives us nearly two hundred on time-dependent properties, a valuable chapter which could usefully have been developed into a book in its own right; and Stephenson, Stanley, Paul and Milosevic close with two chapters on the equilibrium and dynamic properties of fluids at their critical points.

The editor has brought together some first-class players who have performed admirably. He has not, however, welded them into a team, for there is too much repetition and too little evidence that any author knew what the others were saying, except in the two chapters written by the editor himself. Thus one example of this lack of cooperation is that the important function usually written $h(r)$ is called the total, the net and the indirect correlation function in different chapters. The indexing inevitably suffers where there are such discrepancies. No doubt the editor, like others who have undertaken similar tasks, was only too glad to receive the final contribution not too many years behind the original schedule to want to spend more time correlating the material. Certainly the rich meal he has assembled would have been spoilt by keeping. The penalty of concentrating on recent developments is that this book will lose its usefulness more quickly than might be expected from a treatise. It is really a group of timely monographs.

J. S. ROWLINSON

CORRESPONDENCE

Science and Politics

SIR,—Your most recent contributors to the debate on NATO sponsorship display remarkable skill in casuistry. I have just returned from a NATO sponsored International Summer School at the University of Antwerp on Modular Functions of One Variable and Arithmetical Applications; and for me it proved impossible to isolate intellectual interest in the pure mathematics from feelings of disquiet that one was participating in an Institute which could be held to legitimize NATO, or at least to lend its name spurious respectability. After the opening speeches of the conference, the participants were addressed unofficially by A. Grothendieck, one of France's most distinguished mathematicians and a well-known opponent of military involvement in mathematical research. He exhorted us to consider the implications of our attendance, and throughout the lavish reception following, sat among us sending up balloons bearing anti-NATO slogans. This disturbed and angered many of the visitors. Discussion was renewed in the second week with the circulation of a long statement of protest by R. Gode-ment of France, while in the interim a number of participants endeavoured unsuccessfully to arrange a meeting with a NATO spokesman to discuss the aims of their Scientific Affairs Division. Finally, I decided that the only way to atone for my own involvement was to repay the US\$100 grant from the sponsors and never to attend such a conference again.

It seems to me that the Summer School was spoiled from the beginning by its sponsorship. The organizers of such a conference are fully aware that a proportion of the mathematical community cannot allow themselves to accept NATO money, and in this case they had been informed that one of the most important contributors to the subject would not attend if NATO was involved. So the conference suffered badly from his absence and a number of important others. Not to speak of radical students and mathematicians from Communist and Third World countries. A conference supported by non-military funds—and, in fact, a smaller gathering could have been financed with money from the French

CNRS—could have achieved a higher level of scientific excellence and involved mathematicians in a truly international way, regardless of their political beliefs.

Yours faithfully,

STUART W. ELLIOTT

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Department of Pure Mathematics and
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Terrorism in Vietnam

SIR,—In a recent letter (*Nature*, 238, 57; 1972) Dr Lester Goldstein wondered why in your editorial on the "Deadly Irony of Terrorism" (*Nature*, 237, 301; 1972) concerning the tragic and useless death of Professor Aharon Katchalsky Katzir, no mention was made of the kind of terrorism going on in Vietnam. The editor states diplomatically that Vietnam is not part of *Nature's* parish. Does this mean that *Nature* is not concerned with the scientific developments and use of the most sophisticated weapons ever developed, the use of which implicates the responsibility of all scientists? If terrorism has become a routine in Vietnam, it is more than anywhere else because of the silence of responsible people, journals and governments, which is in fact a sign of complicity and acceptance. It is high time that *Nature* faced its responsibilities in this issue.

Yours faithfully,

M. ERRERA

R. THOMAS

*Université Libre de Bruxelles,
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1640 Rhode-St-Genèse*

Bukovsky

SIR,—I don't know how wise it is for *Nature* to be involved in political issues, but I was pleased to see your note on Medvedev's treatment in Kiev. If a man is famous, or such things happen at an international congress, we hear about them from his colleagues. The only other way to get information is

often to receive it from the Soviet Union by people like Vladimir Bukovsky, who was brave enough to send it.

Bukovsky, however, has received a twelve year sentence for sending details which he thinks show that other persons have been unreasonably detained in mental hospitals for political dissent. The documents he openly despatched to the West contained six case histories; they have something in common with the story detailed in Medvedev's book *A Question of Madness*.

Despite the guarantees of the Soviet Constitution it is well known that protest is only possible in the West. Hence with other psychiatrists a statement was produced stating that the documents raised grave doubts and at least what Bukovsky sent should be widely discussed. Since then five of the six persons mentioned have been released, but not General Grizorenko, who is apparently physically unwell and ageing.

It is natural, therefore, that many of us now feel it important to keep attention on the plight of Bukovsky. This unusual man seems quite unconcerned for his own welfare. He continually protests; he openly gave an interview for Western television but he does not seem to have committed any crime. He has, however, suffered repeated exile, compulsory hospitalization and imprisonment for his forthrightness.

Yours faithfully,

F. A. JENNER

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Ehrlich-Maddox

SIR,—I should like to correct an absurdity which somehow crept into my letter to *Nature* (238, 115; 1972) on the Ehrlich-Maddox controversy. I appear to have said: "... much of the resistance to expansion comes from the vested interest of industrialists ...". What I intended to say was, of course, "... much of the resistance to constraints on expansion ...".

Yours faithfully,

ANTHONY WREN

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Obituary

Professor G. D. Preston



PROFESSOR G. D. PRESTON died at Meikle Cottage Hospital, Perthshire, on June 22, 1972. He was 75.

George Dawson Preston was born in 1896, the son of Professor Thomas Preston, FRS, and was educated at Oundle and Caius College, Cambridge. His school and university careers were separated by service in World War I with the 7th Bn, Alexandra Princess of Wales' Own Yorkshire Regiment, service which was ended by a severe leg wound, the effects of which he suffered with admirable fortitude all his life.

After Cambridge, in 1921, he joined the staff of the Metallurgy Division of the National Physical Laboratory, and so began 22 years of fruitful and pioneering work. The techniques of X-ray and electron diffraction were still in their infancy, and he began a systematic application of them to the crystal structures of metals and alloys, working initially with E. A. Owen and later with L. L. Bircumshaw. During his investigations into the structure of alloys, he formulated the idea that the different atoms show clustering or segregation effects (the so-called Guinier-Preston zones) and it was during his detailed study of Laue photographs of aluminium alloys under different cold working and heat treatment conditions that his attention was drawn to the remarkable background scattering on the plates; not just a general diffuse background, but concentrated regions of intensity part of which at least he found to be strongly temperature dependent. The situation was an interesting one. X-ray methods by the beginning of World War II were being extensively used on a wide variety of problems using the Laue or Bragg reflexions. The diffuse background present in all X-ray photographs was

regarded as a nuisance to be minimized by careful experimental technique, although the brilliant theoretical work of Waller had shown what a wealth of valuable information lay in that very background. Unfortunately, Waller's work was not widely known by experimentalists, and, even if known, was not readily interpretable because of its mathematical complexity.

Preston's work sparked off an upsurge of interest in Britain in thermal diffuse scattering, although the principal credit for opening up the field must certainly go to Laval in France, who began a careful investigation into the scattering from various crystals in 1938. Laval's main results, however, were published in such an obscure journal that there can be no doubt that Preston was entirely unaware of his work. In a very short space of time, different groups of workers in Britain, India and the United States all began looking more closely into the phenomenon, although communication difficulties due to wartime conditions meant that they were essentially working independently of each other. Stimulated by the experimental work, various workers redeveloped the fundamental theory, both to simplify the mathematics and to bring out the physical significance. Preston himself gave a qualitative explanation of the effect by assuming that small groups of segregated atoms in the crystal could scatter X-rays independently of the rest of the lattice, and this "geometrical" theory was further developed by W. H. Bragg. The assumption of small independent groups of atoms, however, is hardly reconcilable with our knowledge of the dynamics of crystal lattices, and Max Born showed that the partial agreement with experiment of the simple Preston-Bragg theory was accidental, and that the full Waller theory was necessary for a complete explanation. This work on thermal diffuse scattering was the culmination of Preston's work at the NPL, much of which showed considerable experimental skill. Mounted prints of some of his Laue photographs still elicit admiring comments from workers in the field.

In 1943 Preston left the NPL on his appointment to the Harris chair of physics in University College, Dundee, in the University of St Andrews. During his first few years there, he followed the Scottish tradition of teaching the entire first-year course himself, in addition to giving a normal quota of honours lectures. One curious feature of his teaching will be remembered ruefully by his former students: he had a predilection for quaternion methods, and resolutely

refused to use the Gibbs notation, a refusal which he defended with great wit and persuasiveness. With a teaching load like that, and with the ludicrously small research budget at his disposal, it would not have been surprising if he had given up any idea of continuing with his research work. But in fact X-ray equipment was somehow created from government-surplus junk and work on diffuse scattering was continued. In addition, staff were appointed in other fields of X-ray crystallography, and such work has continued in Dundee to this day. In 1944 he was elected to a Fellowship of the Royal Society of Edinburgh. His tenure of the chair in Dundee saw the beginning and very nearly the end of the long, sometimes bitter, negotiations between St Andrews and Dundee on the future of their relationship. He had little taste for academic intrigue and political bickering, and his dignified and sane attitude throughout the troubled period won him the respect of all his colleagues. He retired in 1966, just one year before St Andrews and Dundee finally separated with the creation of the new University of Dundee, and it was as Professor Emeritus of that institution that he died.

G. D. Preston was a man of wide culture and gentle character, and he will be sadly missed. He is survived by his wife, two sons and two daughters.

Announcements

University News

Dr W. D. Biggs has been appointed to the newly created professorship of building technology in the Faculty of Urban and Regional Studies at the University of Reading.

Professor David Johns has been appointed head of the department of transport technology, **Dr Norman Ashford** to a chair in transport planning, and **Dr Geoffrey Gregory** to a chair in management sciences at the University of Loughborough.

Mr Brian McCloskey has been appointed to the chair of architecture at the Queen's University of Belfast.

Dr D. J. Bartholomew has been appointed to the chair of statistics at the London School of Economics and Political Science.

Dr M. E. H. Turner-Warwick has been appointed to the chair of medicine at the Cardiothoracic Institute, University of London.